

EFFECTS OF THE APPETITE SUPPRESSANT DRUG D-FENFLURAMINE
IN LEAN AND OBESE FEMALE RATS:
CENTRAL AND PERIPHERAL ANTI-OBESITY ACTIONS

By

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Fenfluramine is an appetite suppressant used in the treatment of obesity. Until recently, only the racemate was available clinically and was used in most experimental research. The enantiomers have different properties however, and d-fenfluramine is the more potent anorectic. Isomeride (d-fenfluramine) is available in Europe and is being tested for marketing in the U. S.

There are two major differences in the way fenfluramine is used clinically and in most experimental research. Clinically, fenfluramine is administered in slow release capsules and the metabolism is such that plasma concentrations of the parent compound are always greater than those of the active metabolite norfenfluramine. In rats, fenfluramine is typically administered in single daily injections, often at high dosages. The half-life of fenfluramine in rats is 4-6 hours so the concentration of norfenfluramine is greater than that of fenfluramine within a few hours of administration. Clinically, fenfluramine is administered

to obese patients, usually females. In most experimental research, normal weight male rats are used.

In order to study drug effects in a paradigm that more closely simulates the clinical situation, d-fenfluramine (3 mg/kg/day) was continuously administered via chronically indwelling osmotic minipumps to lean and obese female rats for 28 days. Obesity was induced by feeding a palatable diet or by ovariectomy. Drug effects were also assessed in Chow-fed "normal" weight rats and ovariectomized rats treated with estradiol-benzoate. Effects of d-fenfluramine were compared to dietary restriction and posttreatment effects were measured. Central and peripheral actions were examined.

Fenfluramine suppressed food intake and body weight in all but estradiol-treated rats and was especially effective in ovariectomized rats. This method of administration did not deplete brain 5HT, as did higher dosages in other paradigms.

Drug actions that may be important in appetite suppression and weight loss include chronic stimulation of brain 5HT activity without depletion; decreased motivation to eat or attenuation of rewarding properties of food; effects on thermogenesis evidenced by increased $(\text{Na}^+-\text{K}^+)\text{ATPase}$ activity and cold-stimulated thermogenesis; effects on lipid metabolism including a reduction in lipoprotein lipase activity and effects on carbohydrate metabolism including increased plasma corticosterone and improved glucose tolerance.

CHAPTER I

INTRODUCTION

Obesity and its associated health risks constitute a major health problem. It has been reported that more than 36 million people in the United States are statistically obese (Van Itallie, 1983), while many more are mildly overweight. Strong cultural pressures and social disapproval of obesity have led many individuals (especially women) to adopt a lifestyle of nearly constant dieting. This pattern has increasingly come to include more drastic measures such as periodic starvation, purging and laxative abuse as individuals struggle to achieve or maintain a culturally approved body image.

It has been estimated that as many as 90% of Americans consider themselves overweight. According to a recent marketing survey, about 30% of females and 16% of males in the United States were on diets in 1984 and according to another survey, 16% of the women between ages 19 and 39 considered themselves constant dieters.

The prognosis for long term weight loss is very poor. Even when the obese lose significant amounts of weight by dieting, the loss is rarely maintained (e.g. Blundell and Hill, 1985; Silverstone and Goodall, 1986). A recently reported study involving long term follow up on reduced weight individuals found

that only 15-20% of mildly to moderately obese patients maintained a significant weight loss for 5 years (Pleas, 1986) and the prediction may be even poorer for the morbidly obese.

Across the life span, body weight progressively increases to a maximum level, most often rising in "spurts" interspersed with periods of relative stability. Maximum weight may be maintained for a variable period of time and is normally followed by spontaneous weight loss that may be either rapid or gradual. In most cases, attempts to alter this pattern are only temporarily successful. Dieting, whether it involves food restriction alone or is accompanied by administration of appetite suppressant drugs, typically produces a pattern of relatively rapid weight loss followed by a slowing of the rate of weight loss over a period of about 6 months. Weight loss reaches a plateau and is maintained for a variable length of time followed by a gradual increase to a level at, or above, the initial weight. Both clinical and experimental research suggest that severe or repeated episodes of food restriction (dieting) may actually increase the ease with which weight is regained (Coscina and Dixon, 1983; Pertschuk et al., 1983; Rolls et al., 1980; Shetty et al., 1981). For example, in rats with experimentally imposed fluctuations in body weight (cycling), the efficiency of weight gain increases with each successive episode of diet-induced weight gain (Williams and Senior, 1979). It is also possible that dieting may, in some

people, increase the likelihood of bingeing episodes (Polivy and Herman, 1985; Ruderman, 1986).

Pharmacological intervention in the treatment of obesity began in 1937 when amphetamine was found to reduce food intake in rats (Ehrich and Krumbhaer, cited in Carruba et al., 1986) and in humans (Nathanson, cited in Carruba et al., 1986). The undesirable side effects and high abuse potential of amphetamine encouraged continued research into the development of appetite suppressant drugs. Most of these drugs were derived from amphetamine and share a chemical structure similar to the sympathomimetic amines, with various substitutions on the basic beta-phenylethylamine ring. The most successful of these compounds is fenfluramine (N-ethyl- α -methyl- m -trifluoromethyl-phenylethylamine) which differs from amphetamine structurally by the addition of a halogen (CF_3) group on the ring and an ethyl group on the amine side chain. The pharmacological profile of fenfluramine is dissimilar from amphetamine in that it lacks psychomotor stimulant properties, has very low abuse potential and appears to act primarily on brain serotonin (5-hydroxytryptamine [5-HT]) rather than catecholamines (Pinder et al., 1975).

The plasma half-life of d-fenfluramine is approximately 24 hours in humans and 4 hours in rats (Caccia et al., 1982). The most active pathway of metabolism involves the rapid dealkylation of fenfluramine to the active metabolite norfenfluramine (Beckett

and Brookes, 1970; Bruce and Maynard, 1968) which has a plasma half-life of 48 hours (Beckett and Salmon, 1972; Campbell et al., 1979). Fenfluramine and norfenfluramine are converted more slowly to 3-trifluoromethyl-benzoic acid and then to the glycine conjugate 3-trifluoromethyl-hippuric acid which is excreted in the urine (Beckett and Salmon, 1972; LeDouarec and Neveu, 1970).

Fenfluramine exists in both d- and l-enantiomers. Until recently, only the racemate was available clinically and was used in most animal research. The d- and l-enantiomers have different properties however, and the d-isomer is a much more potent anorectic in animals (Borsini et al., 1982) and in humans (Silverstone and Goodall, 1986). Dextrofenfluramine (Isomeride) is available by prescription in Europe and is currently being tested for marketing in the United States.

Research on the pharmacology of drugs that affect appetite and body weight has a two-fold purpose: (a) in the development of more effective treatments for eating disorders and obesity and (b) as tools to investigate mechanisms of regulation of food intake and body weight.

The relevance of animal studies to clinical applications in eating and weight disorders has been questioned seriously in recent years. There are several important ways in which procedures for animal research on the pharmacological actions of fenfluramine often differ from the clinical setting. The animal

models most commonly used in the study of ingestive behavior involve structured feeding schedules or food deprivation paradigms in normal weight laboratory rats fed a uniform, bland diet. It is not surprising that the results from these experiments do not closely match the eating patterns of obese humans. In the studies reported here, animal models of obesity that incorporate factors relevant to human obesity were selected. A range of behavioral, as well as both central and peripheral biochemical variables, and their interactions were examined in order to develop an experimental situation that would be objectively and conceptually more similar to that seen clinically in humans.

Clinically fenfluramine is administered in slow-release capsules or tablets taken twice daily. The dosage is gradually increased during the first weeks of treatment to approximately 1-2 mg/kg/day d,l- and 0.5-1 mg/kg/day d-fenfluramine. There is a gradual accumulation of drug in adipose and other tissues until steady-state plasma levels are reached (Campbell et al., 1979).

Although the half-life of norfenfluramine is approximately twice that of fenfluramine, drug metabolism in humans is such that the concentration of the parent compound is generally greater than that of the metabolite. In rats however, plasma concentrations of the metabolite are relatively greater within 2-6 hours of administration (Caccia et al., 1982). Norfenfluramine, unlike its ethylated parent, appears to have direct actions at 5-HT

postsynaptic receptors (Borsini et al., 1982; Mennini et al., 1985). When d,l-fenfluramine is administered, the l-isomer is preferentially metabolized in humans (Beckett and Brookes, 1970) whereas the d-isomer is metabolized faster in rats (Morgan et al., 1972). Thus the more active isomer is more quickly eliminated in rats, but not in humans.

Much of the information on both the central and peripheral actions of fenfluramine has been obtained from in vitro preparations using high drug concentrations. Studies conducted in vivo have typically used one- or two-daily injections of a single drug dosage that is often well above the ED_{50} for producing anorexia in the species being tested. In previous experiments, we have noted that rats treated with d,l-fenfluramine, at a dose range of 10 mg/kg or greater, behave in a manner that is strikingly different from rats given lower dosages. In reviewing the literature, one finds that the vast majority of studies on the biochemical actions of both d- and d,l-fenfluramine report using relatively high drug dosages (≥ 7.5 mg/kg d- or ≥ 10 mg/kg d,l-fenfluramine). In the cases where a range of drug dosages is tested, effects at high dosages often are not found at the lower dosages.

It is possible that procedures using intermittent administration of a constant, high drug dosage produce effects that are not strictly applicable to the clinical situation. This may be a reason for some of the discrepancies between clinical and

experimental observations. In order to model more closely a clinical treatment regimen, d-fenfluramine was administered continuously via chronically indwelling osmotic pumps that delivered 3 mg/kg/day at a rate of 0.5 ul/hour. Considering the difference in the plasma half-life of fenfluramine, this dosage is comparable to the customary clinical dosage of 0.5 mg/kg/day.

CHAPTER II

LITERATURE REVIEW

Overview of the Antiobesity Drug Fenfluramine

Effects of Fenfluramine on Food Intake and Body Weight of Humans and Experimental Animals

Fenfluramine is effective in producing weight loss in obese humans (Burland, 1975; Innes et al., 1977; Munro and Ford, 1981). At a dose of 60-120 mg/day (vis. 1-2 mg/kg/day), d,l-fenfluramine produces an average weight loss of approximately 10 kg over a period of 6 months (Innes et al., 1977; Stunkard, 1981). Body weight is maintained at a lowered level until drug therapy is discontinued. Weight is regained within about 12 months of termination of drug treatment (Douglas et al., 1983; Stunkard, 1981). Fenfluramine is lipid soluble and accumulates in adipose tissue so that steady plasma levels are attained within several days of treatment initiation (Rowland and Carlton, 1986).

In normal weight animals fed laboratory Chow on a 4 hour/day feeding schedule, a dosage of 5 mg/kg d,l-fenfluramine acutely suppresses food intake by approximately 80%. Thereafter, anorectic tolerance develops rapidly and food intake returns to baseline levels within 5-7 days of chronic administration (Antelman et al., 1981; Ghosh and Parvathy, 1976; Goudie et al., 1974; Rowland et al., 1983). A similar tolerance curve is obtained in paradigms that do not involve food deprivation such as "dessert"

tests (Carlton and Rowland, 1984) and tail pressure-induced eating (Antelman et al., 1981). Tolerance to d-fenfluramine resembles that seen with the racemate; however, the d-isomer is much more potent and tolerance may be delayed or incomplete with doses of 5 mg/kg or greater (Rowland and Carlton, 1986).

The effects of d- and d,l-fenfluramine on the body weights of normal weight animals parallel its anorectic effects: there is an initial dose-dependent weight loss followed by weight gain at a trajectory parallel to controls (eg. Brindley et al., 1985; Duhault et al., 1979). In obese animals, anorexia may be prolonged and weight loss sustained over longer periods of time with either d,l- or d-fenfluramine (Carlton and Rowland, 1986; Rowland and Carlton, 1986).

Data compiled from several experiments conducted in our laboratory, using diverse groups of rats (initial body weights ranging from 200-500 g), revealed a significant correlation between initial body weight and absolute weight change after ten daily injections of 5 mg/kg d,l-fenfluramine (Rowland and Carlton, 1986). Rats with initial body weights less than 280 g gained weight during d,l-fenfluramine treatment, albeit at a slower rate than saline-injected controls, whereas rats initially weighing more than 280 g sustained an absolute weight loss.

Several lines of evidence suggest that fenfluramine produces sustained weight loss in obese humans and laboratory animals by

antiobesity actions that may be independent of appetite suppression: (a) the maintenance of weight loss until termination of drug therapy, followed by rebound weight gain seen clinically; (b) sustained weight loss accompanied by anorectic tolerance found under certain experimental conditions; (c) increased drug efficacy in obese versus lean animals and (d) enhanced loss of body fat in obese women.

Antiobesity Actions of Fenfluramine: A Set-Point Model

The increased drug efficacy in obese relative to lean animals has been interpreted in terms of a set-point model (Stunkard 1986). According to Stunkard's theory, fenfluramine acts to lower the level at which body weight is regulated, resulting in decreased food intake and concomitant weight loss until the new level is reached.

Levitsky et al. (1981) proposed that the apparent anorectic tolerance to d,l-fenfluramine that develops in laboratory animals is due to increasing deprivation and weight loss rather than a true biological drug tolerance. This conclusion was based on a study in which those authors reported that rats with experimentally induced body weight reductions showed no anorexia compared to normal weight rats when treated acutely with a high dose of d,l-fenfluramine (20 mg/kg). The interpretation of those results has not gone unchallenged (Carlton and Rowland, 1985, Rowland and Carlton, 1986); however, that study, along with the clinically

observed weight "rebound" that often occurs upon termination of d,l-fenfluramine treatment, formed the basis for Stunkard's set-point model. This model has been applied to the actions of both d- and d,l-fenfluramine.

There are two aspects of the set-point model that are relevant here: one concerning anorexia and weight loss and the other concerning tolerance to these effects. Homeostatic set-point models are based on the premise that a given physiological variable (e.g. body weight, body temperature, blood glucose) is regulated within a set range and that deviations from this range are detected. Subsequent behavioral and/or physiological responses are aimed at restoring the variable to its original level. According to Stunkard's set-point model, fenfluramine initially acts to lower the level at which body weight is regulated. This results in decreased food intake and weight loss until the new level is reached. The new level is maintained and presumably defended, as long as drug treatment is continued.

The set-point model has also been used to explain the apparent development of tolerance to d,l-fenfluramine-induced anorexia that occurs in most animal studies (Levitsky et al., 1981; Stunkard, 1981). Accordingly, it was suggested that changes in nutritional status resulting from drug-induced food deprivation and/or reduced body weight increase motivation to eat and hence produce an apparent anorectic tolerance. This line of reasoning

has been extended to include clinical observations. Thus it was suggested that obese humans do not develop tolerance to d,l-fenfluramine-induced anorexia and weight loss as quickly as experimental animals because their nutritional reserves are large enough that the relevant period of food deprivation does not lead to reduced anorectic potency.

We have tested the set-point theory of anorectic tolerance to d,l-fenfluramine using what we considered to be a more thorough experimental design (Carlton and Rowland, 1985). We adopted a paradigm similar to the one used by Powley and Keesey (1970) in the now classical experiments in which they found that prior starvation prevented the aphagia that ordinarily occurs in rats subsequent to lateral hypothalamic knife-cuts. We hypothesized that if tolerance to d,l-fenfluramine anorexia is an artifact of weight loss, pretreatment deprivation should prevent acute anorexia in a similar manner.

Three groups of rats were placed on restricted feeding schedules of variable length to produce weight losses of approximately 10, 20 or 30% relative to controls with ad libitum food access. When body weights became stable at the experimentally reduced levels, we began drug treatment. The initial administration of d,l-fenfluramine (5 mg/kg i.p.) effectively suppressed food intake to a similar degree in each of the four groups. All groups were returned to ad libitum feeding and daily drug

injections were continued for 16 days during which anorectic tolerance developed at a similar rate in all groups.

The results of the preceding experiment are in apparent opposition to the hypothesis that d,l-fenfluramine acts primarily to lower body weight set-point. Moreover, tolerance to d,l-fenfluramine anorexia develops in paradigms that do not involve deprivation or weight loss such as eating in response to tail pinch (Antelman et al., 1979), muscimol-stimulated eating (Borsini et al., 1983) and "dessert" tests (Carlton and Rowland, 1984). Clearly, a simple model of set-point regulation cannot be universally applied to explain all of the observed effects of fenfluramine; however, the previous arguments are not sufficient to categorically refute the validity of the set-point concept.

The preceding studies have used alterations in food intake as an indicator of changes in body weight set-point. Inferences about set-point derived from changes in body weight are tautological and so even less convincing. Fantino et al. (1986) recently have proposed a more powerful model for understanding body weight regulation and set-point in rats based on hoarding behavior. Rats normally do not hoard food when fed ad libitum, but begin to do so when they lose weight from food deprivation. In Chow-fed rats, hoarding begins following a weight loss of less than 10 g and the amount hoarded is proportional to weight loss. A loss of 30% body weight results in intense hoarding behavior that decreases in

proportion to restoration of body weight. According to this model the body weight set-point is the weight at which the amount hoarded reaches zero on the line of regression for amount hoarded versus body weight.

This model has been used to study dietary obesity and the effects of d-fenfluramine (Fantino et al., 1986). Hoarding behavior in Chow-fed rats treated with d-fenfluramine did not begin until the animals lost 60 g, thus supporting the notion that d-fenfluramine lowers body weight set-point. Rats fed a cafeteria diet did not begin hoarding until they had lost 50 g, suggesting that body weight was maintained above set-point levels. The effects of a palatable diet sustained eating above the set-point regulated level. Dextrofenfluramine further increased the amount of weight loss necessary to invoke hoarding behavior in rats with diet-induced obesity (DIO) to 120 g.

The level at which body weight is regulated in DIO rats may depend upon the duration of the diet. Dietary obesity is reversible up to a point after which it may become permanent. Brief exposure to a palatable diet results in an increase in fat cell size whereas longer exposure increases cell number (Faust et al., 1978). When the number of fat cells increases, obesity is more difficult to reverse and body weight is regulated at a higher set-point.

Effects of Fenfluramine on Brain 5-Hydroxytryptamine (5-HT)

Central actions of fenfluramine, particularly those involving brain 5-HT, have been studied extensively. Until recently, the majority of behavioral studies have assumed that observed drug effects were primarily, if not exclusively, mediated by brain 5-HT. Blundell (1977, 1979) has published reviews on the involvement of brain 5-HT in the regulation of feeding behavior. The precise nature of this role and the pathways involved have not been defined; however, it is believed to be generally inhibitory. Continued research on the effects of fenfluramine has yielded results that are not readily explainable in terms of a singular brain 5-HT mechanism. In the last 2-3 years, these inconsistencies have become increasingly difficult to ignore.

Leibowitz and Shor-Posner (1986) have reported a dose-dependent inhibition of food intake after injection of 5-HT or norfenfluramine into the paraventricular nucleus (PVN) of the hypothalamus. Moreover, the observed effects on the microstructure of feeding were similar to the effects of peripherally administered fenfluramine (vis. a slowing of the rate of feeding and early termination of meals). Whereas these results seem to suggest a central mode of action, d,l-fenfluramine has a low potency when administered centrally by intracerebroventricular (icv.) infusion (Rowland and Carlton, 1986) or by direct injection into the hypothalamus arguing against a primary central action (Davies et al., 1983).

Traditionally, feeding research has focused on the hypothalamus as a center for the control of ingestive behaviors. The early theories of excitatory and inhibitory "control centers" located in the lateral and ventromedial hypothalamus have given way to increasingly precise neuroanatomical localization. Whereas it is conceptually similar to control center theories, the work of Leibowitz and her colleagues has systematically reduced the focus of pharmacological studies to comparatively minute brain regions. This is a potentially powerful approach that should contribute significantly toward explaining apparent inconsistencies and confusing results.

Fenfluramine competitively inhibits [^3H]5-HT reuptake by brain synaptosomes and stimulates its release from vesicular storage sites (Fuxe et al., 1975; Garattini et al., 1979). The d- and l-enantiomers stimulate release with approximately equal potency (Duhault and Verdavainne, 1967; Garattini et al., 1975; Ghezzi et al., 1973). Dextrofenfluramine is approximately 10-times more potent in inhibiting uptake of [^3H]5-HT than is the l-isomer (Garattini et al., 1979; Mennini et al., 1985). Anorectic potency is thus correlated with inhibition of reuptake (Mennini et al., 1985); however, inhibition of 5-HT reuptake alone may not be sufficient to produce anorexia (Samanin et al., 1980a) or may do so by changing meal frequency rather than meal size (Burton et al., 1981).

Chronic administration of moderate dosages of d,l-fenfluramine (<10 mg/kg/day) and acute administration of higher dosages of d- or d,l-fenfluramine are associated with decreased brain levels of 5-HT and 5-HIAA in rats (Duhault and Verdavainne, 1967; Duhault et al., 1979, 1981; Garattini et al., 1979). Depletion of 5-HT begins within the first 1-2 hours of drug administration and is followed by a decrease in 5-HIAA (Duhault and Verdavainne, 1967; Duhault et al., 1980; Fuller et al., 1978). This suggests that 5-HT is depleted due to increased release without a compensatory increase in synthesis.

The rate of 5-HT synthesis in brain is normally limited by the availability of the precursor amino acid tryptophan. Acutely administered d,l-fenfluramine has been reported to increase (Tagliamonte et al., 1971) or have no effect (Costa et al., 1971) on brain levels of tryptophan; however, the rate of 5-HT synthesis measured by accumulation of the immediate precursor 5-hydroxytryptophan (5-HTP) following inhibition of 5-HTP decarboxylase is decreased by acute d,l-fenfluramine.

Decreases in synthesis are found as early as 2 hours after administration of 10 mg/kg d,l-fenfluramine (Rowland and Carlton, 1986) and may last up to 8 days after a single dose of 15 mg/kg d,l-fenfluramine (Clineschmidt et al., 1978). The ratio of 5-HIAA/5-HT is initially increased (Fuller et al., 1978; Orosco et al., 1984) which further suggests increased neuronal activity;

however, it is not clear whether this increased activity is sustained throughout the period of drug administration.

Decreases in whole brain 5-HT produced by high doses of d,l-fenfluramine are still evident at one month after acute administration (Clineschmidt et al., 1978) although most of the drug is cleared within 24 hours (Garattini et al., 1979). This long-lasting depletion may be due to a neurotoxic action on the nerve terminal (Fuller, 1981). The binding of [¹⁴C]fenfluramine to brain tissue preparations (Belin et al., 1976; Duhault et al., 1980) implies that fenfluramine can bind to 5-HT receptors. Thus, the fenfluramine-stimulated release of 5-HT may be dependent upon binding of the drug to presynaptic receptors (Rowland and Carlton, 1986). Depletion of brain 5-HT can be prevented by prior administration of drugs that block 5-HT reuptake (Clineschmidt et al., 1978; Fuxe et al., 1975) and so, apparently requires the uptake of fenfluramine into the nerve terminal.

Both fenfluramine and norfenfluramine inhibit the in vitro binding of [³H]5-HT to rat brain membranes. The efficacy of the l-enantiomers ($IC_{50} = 2 \text{ } \mu\text{mol}$) is double that of d-norfenfluramine ($IC_{50} = 4 \text{ } \mu\text{mol}$) and more than 3-times greater than that of d-fenfluramine ($IC_{50} = 7 \text{ } \mu\text{mol}$) (Mennini et al., 1985).

Changes in the number of [³H]5-HT binding sites (B_{max}) have been reported following procedures that either increase or decrease central 5-HT availability (Hamon et al., 1980). A

decrease in the number of [^3H]5-HT binding sites in rat frontal cortex has been reported following 28 days, but not 14 days, of chronic d-fenfluramine administration (2.5 mg/kg twice daily) (Samanin et al., 1980b). This was originally interpreted as evidence that subsensitivity of 5-HT receptors may underlie the development of anorectic tolerance; however, the discrepancy between the time courses for the reported biochemical changes and the development of anorectic tolerance suggests that these phenomena are not functionally related. We have been unable to confirm those observations and have not found consistent changes in binding of [^3H]5-HT (putative S_1 receptors) or [^3H]spiroperidol (putative S_2 receptors) to membrane preparations from various brain regions following chronic d,l-fenfluramine (Rowland et al., 1983).

If peripherally administered fenfluramine exerts its anorectic effects via enhanced central 5-HT transmission, one would expect that injection directly into brain tissue or cerebrospinal fluid would be similarly effective. Broekkamp et al. (1975) first reported that d,l-norfenfluramine administered into the stria terminalis reduced food intake of food deprived rats, but equal doses of d,l-fenfluramine were considerably less effective. Kruk (1973) showed that icv. injection of d,l-norfenfluramine was also effective; however, those studies used high drug doses to produce modest effects on food intake (Davies et al., 1983, Rowland and Carlton, 1986).

The 5-HT receptor blocker metergoline reliably reverses d,l-fenfluramine-induced anorexia when both are administered peripherally (Clineschmidt et al., 1974); however, peripherally administered metergoline does not block anorexia produced by centrally administered d,l-fenfluramine (Rowland and Carlton, 1986). This strongly suggests that peripherally administered fenfluramine does not act exclusively via central 5-HT. Anorexia produced by centrally administered d,l-fenfluramine or norfenfluramine can be partially overcome by tail pressure stress whereas the anorexia produced by peripheral administration is not affected (Antelman et al., 1979, 1981), again suggesting disparate mechanisms.

Another way to examine the role of central 5-HT in mediating fenfluramine's actions is to deplete brain 5-HT prior to drug administration. If the primary action of fenfluramine is via its effects on release and uptake of 5-HT at presynaptic terminals, damage to these terminals resulting from electrolytic or neurotoxic lesions should reduce the efficacy of fenfluramine. Rowland and Carlton (1986) reviewed the effects of various techniques for producing brain 5-HT depletions on d,l-fenfluramine-induced anorexia in rats. A table listing the results (p. 47) showed that anorexia was attenuated in four cases, enhanced in four cases and not affected in three cases (two of the studies reported results on two groups). We had previously reported that substantial

depletions of brain 5-HT, induced by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), resulted in a slight increase in the efficacy of 2 and 5 mg d,l-fenfluramine/kg and a decrease in the efficacy of 10 mg/kg (Carlton and Rowland, 1984).

Effects of Fenfluramine on Brain Reward Systems

Effects on brain opioids. Both central and peripheral opioids may be involved in feeding behavior and energy regulation (Majeed et al., 1986; reviewed in Morley et al., 1983). There is also evidence for interactions between brain opioid and monoamine systems, the exact nature of which is unclear and may be reciprocal (Dellavedova et al., 1982). Exogenous administration of opioids increase 5-HT synthesis and release (Smialowska and Bal, 1984) and changes in hypothalamic and striatal 5-HT activity are associated with changes in opioid levels in these areas (Groppetti et al., 1984; Harsing et al., 1982). Depletion of brain 5-HT has been reported to increase brain opioid levels (Han et al., 1980). This probably reflects neuropeptide accumulation due to decreased utilization rather than increased synthesis (Mocchetti et al., 1985).

There is evidence that brain opioid and 5-HT system interactions may be influenced by fenfluramine and in turn, may affect anorexia and/or tolerance. Administration of d-fenfluramine (15 mg/kg/day for 5 days) has been associated with increases in hypothalamic concentrations of met-enkephalin and beta-endorphin

that last up to 10 days after termination of drug administration (Harsing et al., 1982). Those authors suggested that the anorectic effects of d-fenfluramine may be mediated by decreased opioid activity. Others (Groppetti et al., 1984) have proposed that the fenfluramine-induced release of brain 5-HT is modulated by the opioids and that anorexia is dependent upon 5-HT release. Following depletion of 5-HT with a single large dose or repeated small doses of d,l-fenfluramine, acutely anorectic doses of the drug were not effective in further depleting 5-HT and did not produce anorexia. Increased hypothalamic and striatal met-enkephalin were also associated with decreased 5-HT in that study. Depletion of 5-HT was potentiated by pretreatment with morphine and antagonized by naloxone. In d,l-fenfluramine-tolerant rats, morphine pretreatment restored the ability of fenfluramine to further deplete 5-HT and to produce anorexia (Groppetti et al., 1984).

Effects on brain dopamine. Fenfluramine increases concentrations of the extraneuronal dopamine (DA) metabolite homovanillic acid (HVA) in the striatum which presumably reflects increased DA release. Tolerance to this effect is not apparent, at least within the time period for anorectic tolerance (Jori and Bernardi, 1972; Jori and Dolfini, 1977). The l-isomer is considerably more potent in stimulating DA activity; however, the d-isomer is also effective in this regard (Jori and Dolfini, 1974; Garattini et

al., 1975). The effect of d,l-fenfluramine on striatal DA turnover is blocked by pretreatment with DA receptor antagonists and therefore appears to be a result of receptor blockade (Crunelli et al., 1980). Although DA antagonists such as neuroleptics can reduce food intake (eg. Rowland and Engle, 1977), this is not thought to be a primary mechanism of fenfluramine anorexia. The possibility of interactions among DA, 5-HT and opioid systems in the central control of food intake, and in the actions of fenfluramine, cannot be dismissed. Opioid and DA systems have been implicated in stress-induced eating and may also be important in mediating the rewarding effects of food.

Peripheral Antiobesity Actions of Fenfluramine

Fenfluramine may be more effective in producing sustained weight loss in animals with a higher percentage body fat. Comparing data from a number of different studies, we have found a correlation between weight loss and initial body weight with chronic daily drug injections (Rowland and Carlton, 1986). In preliminary studies, we found prolonged anorexia and weight loss in animals made obese by feeding a palatable and varied diet. It has been reported that as much 90% of the weight gain observed on similar diets is due to increased lipid storage (Rothwell and Stock, 1979b). We also have indications that fenfluramine may be more effective in female rats than in males. In rats, as in humans, females generally have higher ratios of adipose to muscle

tissue (AMR) than males of comparable body weight. These results suggest that a higher percentage of body fat, rather than increased body weight per se, may be the important factor for explaining the greater efficacy of fenfluramine in heavier animals.

Peripheral actions of fenfluramine may be relevant to its antiobesity actions and may also help explain possible differences in drug efficacy in lean and obese animals. The majority of these studies have been conducted using d,l-fenfluramine, but some of the more recent ones have used d-fenfluramine. Effects of d,l-fenfluramine on carbohydrate metabolism are found at drug concentrations that are clinically relevant. These include (a) increased glucose uptake (Butterfield and Whichelow, 1968) and production of lactate (Kirby and Turner, 1976) suggesting that relatively inefficient anaerobic glucose metabolism is stimulated; (b) increased insulin receptor sensitivity (Verdy et al., 1983) and (c) improved glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970).

Effects of d,l-fenfluramine on lipid metabolism have been studied primarily in vitro using high drug concentrations; therefore, the relevance of these actions to anorexia and weight loss is questionable. These effects include (a) a decrease in total plasma lipids (Pawan, 1970); (b) a decrease in the meal-related rise in plasma triglycerides (Bizzi et al., 1973; Curtis-Prior et al., 1980; Garattini et al., 1975); (c) an acute increase in

circulating fatty acids (FFA), ketones and glycerol (Pawan, 1970) and (d) inhibition of lipogenesis in vitro and in vivo (Wilson and Galton, 1971; Comai et al., 1978) that may be related to lower insulin binding in adipocytes (Harrison et al., 1975). Nicolaidis and Even (1986) have reported a prolonged stimulation of lipolysis and enhanced utilization of lipid substrates using d-fenfluramine (7.5 mg/kg).

Acutely, d,l-fenfluramine stimulates corticosterone release (Fuller et al., 1981; McElroy et al., 1984; Schettini et al., 1979) and has mild sympathomimetic actions including increased plasma norepinephrine (NE) (Calderini et al., 1975; Lake et al., 1979); inhibition of insulin release and stimulation of glucagon release (Barseghian et al., 1983). These effects may be mediated in part by central or peripheral 5-HT: exogenously administered 5-HT stimulates sympathetic nervous system activity (Stajarne and Schapiro, 1959; de Groat and Volle, 1966) and administration of 5-HTP provokes release of insulin and glucagon in vivo (Jacoby and Bryce, 1978). Acute and chronic drug effects may differ. For example, chronic administration of d-fenfluramine decreases the stress-evoked rise in concentrations of corticosterone and catecholamines (Brindley et al., 1985), and decreases in plasma NE and other indicators of sympathetic activity are found with chronically administered d,l-fenfluramine (Lake et al., 1979; Rothwell et al., 1982).

Large doses of d,l-fenfluramine chronically administered increase GDP-binding to brown adipose tissue (BAT) mitochondria (Bray and Lupien, 1984; Lupien and Bray, 1985) which presumably reflects increased thermogenic activity. This effect is not seen with lower doses and others have reported that d-fenfluramine has no effect on resting metabolic rate although the energy cost of locomotor activity may be increased (Nicolaidis and Even, 1986) as well as the thermic effect of food (Levitsky, 1986).

The relevance of any of the central or peripheral actions of fenfluramine to appetite suppression and weight loss is not completely clear. When various actions are viewed in isolation or when effects of single doses and high concentrations of drug are extrapolated to explain clinical actions, the results may seem confusing and contradictory. Only by putting together the series of actions as they occur within a relevant treatment model will it be possible to determine the exact nature of the factors and interactions important to appetite regulation and weight loss.

The Autonomic Nervous System and Hormonal Regulation of Metabolism

Regulation of metabolism is primarily under the control of the autonomic nervous system which regulates the reciprocal processes of energy storage and utilization. Activity of the parasympathetic nervous system stimulates release of insulin and inhibits glucagon release from the endocrine pancreas whereas activation of the sympathetic nervous system has opposite

effects. These hormones in turn have direct and in general, opposing actions at the level of cellular metabolism. The pituitary-adrenal system also has important actions in the regulation of metabolism particularly in times of physiological or psychological stress. The adrenocorticoids have actions similar to the sympathetic neurotransmitters as well as direct effects at the cellular level. The gonadal hormones also have important modulatory actions on metabolism.

Effects of Insulin on Glucose and Lipid Metabolism.

Insulin is released in response to high plasma levels of nutrients such as after a meal. It stimulates glucose utilization and storage as glycogen in liver and muscle. Insulin also stimulates uptake and storage of FFA. Hyperinsulinemia is commonly found in a majority of obese animal models and in humans with longstanding obesity. The result of persistent hyperinsulinemia associated with obesity may be the development of insulin receptor insensitivity and Type II diabetes mellitus which constitutes a major health risk for the chronically obese.

Baseline insulin levels of rats made obese by feeding a high fat, high carbohydrate diet have been reported to be up to 6-times greater than in Chow-fed animals (Triscari et al., 1985). Hyperinsulinemia was not found in hyperphagic rats on similar diets that did not gain weight (Rothwell and Stock, 1981; Levin et al., 1983). Thus, increased body weight rather than caloric intake may

be responsible for hyperinsulinemia or conversely, hyperinsulinemia may be necessary for excessive weight gain. There are species differences in insulin response to diet that may be related to differences in weight gain. Male Sprague-Dawley rats did not gain weight and had normal insulin levels on the high fat, high carbohydrate diet used by Levin et al. (1983); however, a palatable, high carbohydrate diet that we used in preliminary experiments caused most female Sprague-Dawley rats to gain substantial amounts of weight. In contrast to studies in which cafeteria fed rats did not gain significant amounts of weight despite excessive intake (Rothwell and Stock, 1979a, 1979b, 1980; Swann, 1984a), Triscari et al. (1985) found a 3-fold increase in efficiency of energy utilization in rats made obese on their high fat, high carbohydrate diet (cf. Levin et al., 1987). After 3 months on that diet, rats needed significantly less calories to add a gram of body weight suggesting that initially increased energy expenditure may be eventually overridden by mechanisms that conserve energy.

Rats with diet-induced obesity have been reported to have elevated FFA, glycerol and triglycerides. After 3 months on the diet used by Triscari and coworkers, FFA were increased by 38%; glycerol was increased by 41%; triglycerides were 80% above normal and ketogenesis was doubled (Triscari et al., 1985). These elevations in plasma lipids may be related to hyperinsulinemia. The

in vivo perfusion of physiological concentrations of FFA over isolated liver inhibits insulin clearance. This effect is long lasting and occurs in repeated experiments (Smith, 1985). Elevated FFA levels have also been found to inhibit peripheral glucose uptake.

At relatively high doses, d,l-fenfluramine and d,l-norfenfluramine inhibit lipogenesis in adipose tissue. This has been found in rat internal adipose tissue (epididymal) with 1 mmol concentrations of drug (Dannenburg and Kardian, 1970) and in human subcutaneous adipose tissue with 3 mmol drug (Ashwell, 1974; Wilson and Galton, 1971). Plasma FFA concentrations are increased by d,l-fenfluramine in fed, but not in fasted, animals (Barrett, 1964; Chandler et al., 1970). With chronically administered d,l-fenfluramine, plasma FFA levels have been found to be directly correlated with drug levels (Chandler et al., 1970). In some cases, d,l-fenfluramine has been reported to inhibit the lipolytic response of adipocytes to catecholamines (Dannenburg and Kardian, 1969), but this effect has not been found in other studies (e.g. Finger et al., 1966).

The insulin-dependent uptake of glucose in muscle is stimulated by d,l-fenfluramine and this appears to be mediated by 5-HT (Kirby and Turner, 1976; Turner, 1979; Turner et al., 1982). It also reduces fasting plasma glucose levels and improves glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and

Butterfield, 1970), effects that are not mediated by increased insulin release (Pasquine and Thenen, 1981). In vitro, high concentrations of d,l-fenfluramine (1 mmol) reduce the glucose-stimulated insulin release from perfused rat pancreas. This effect is not 5-HT dependent and is not found with lower drug concentrations (Barseghian et al., 1983). Fenfluramine-stimulated increases in glucose uptake may be mediated by increased insulin receptor sensitivity (Verdy et al., 1983) and/or by directly binding to receptors (Harrison et al., 1975).

The chronic effects of d,l-fenfluramine on insulin release are equivocal. In obese women treated chronically with 60 mg of d,l-fenfluramine given in three doses per day, a test dose of 40 mg suppressed insulin levels. Lowered insulin levels also have been reported in Type II diabetics (Asmal and Leary, 1975). However, a dose of 20 mg d,l-fenfluramine had no effect on insulin levels of normal weight, healthy subjects (Sulaiman and Johnson, 1973). The issues of drug dose and obese versus nonobese subjects may be relevant. At high concentrations, d,l-fenfluramine inhibits ketogenesis and also inhibits gluconeogenesis from lactate, pyruvate and alanine (Geelen, 1983).

Effects of Glucocorticoids on Metabolism and Body Weight

The glucocorticoids oppose the actions of insulin on gluconeogenesis, glycolysis, protein synthesis and glucose uptake in some tissues and decrease insulin sensitivity; however, they facilitate

the actions of insulin in stimulating synthesis of glycogen and FFA (Amantruda et al., 1983) and increasing activity of the enzyme lipoprotein lipase (LPL) in adipose tissue (Robinson et al., 1985). In stress conditions, lipolysis is stimulated and circulating FFA are increased. The increased availability of FFA, along with possible direct stimulation of hepatic phosphatidate phosphohydrolase, increase synthesis and secretion of triglycerides (Brindley, 1983).

Exogenously administered glucocorticoids cause decreased appetite and weight loss reflected in both protein and lipid loss. When insulin is simultaneously administered, lipid storage and body weight are increased although protein wasting persists (Hausberger and Hausberger, 1958). This is similar to the clinical picture of Cushing's Syndrome in which protein wasting is associated with increased circulating glucose and high insulin levels that in turn promote excessive lipid storage. The consequences of this are truncal obesity, impaired glucose tolerance, diabetes and hypertension. In longstanding obesity, circulating corticosterone levels may be increased along with increased insulin levels and this may be associated with the development of android obesity (Vague, 1983).

Hypercorticism is common to several animal models of obesity including hypothalamic, genetic and diet-induced obesity. Adrenalectomy reverses or prevents the development of obesity by

decreasing food intake and energy efficiency, restoring insulin sensitivity and glucose tolerance to normal and increasing GDP-binding in BAT (Bray, 1985).

The glucocorticoids also affect insulin sensitivity. Injection of ACTH or glucocorticoids can produce a syndrome of insulin resistant diabetes. Glucose intolerance is also observed in Cushing's syndrome and in patients undergoing glucocorticoid therapy (Munck, 1971; Pupo et al., 1966). This appears to be due to a decrease in receptor affinity for insulin and similarly, adrenalectomy causes an increase in insulin receptor affinity (Kahn et al., 1978). It is known that d,l-fenfluramine can affect glucose metabolism and insulin sensitivity at clinically relevant doses; however, its effects on insulin and glucocorticoids and the relationship to changes in 5-HT activity are not well understood.

Adrenocorticotrophic hormone (ACTH), which stimulates production and release of adrenal glucocorticoids, and beta-lipotropin, the precursor of beta-endorphin and melanin stimulating hormone, are produced from the same pro-hormone (pro-opiomelanocortin) and may be released simultaneously (Dubuc et al., 1975). Beta-endorphin has been shown to stimulate glucose-dependent insulin release (Ipp et al., 1978). Under conditions of prolonged stress, and perhaps as a natural consequence of aging, production and release of ACTH and beta-endorphin are increased. Thus, it has been suggested that this combination of glucocorticoid-stimulated

gluconeogenesis and beta-endorphin enhancement of insulin release results in increased lipid storage relative to caloric intake (Margules, 1979).

Acutely, d,l-fenfluramine stimulates the release of adrenal glucocorticoids and catecholamines (Schettini et al., 1979) and stimulates lipolysis in adipose tissue (Dannenburg, 1983). Brindley (1983) has found similar effects with d-fenfluramine. These effects appear to be mediated by increased hypothalamic 5-HT availability: 5-HT stimulates release of corticotropin releasing hormone (CRH). The time course of this action is parallel to that of d,l-fenfluramine-induced anorexia (Fuller et al, 1981). The physiological role of the 5-HT-stimulated CRH release is not clear; however, it appears to be involved in diurnal rhythmicity of adrenocortical activity (Fuller, 1981) that in turn is correlated with feeding patterns in rats (Dallman, 1984). The role of this pathway in mediating acute stress responses is questionable: inhibition of 5-HT uptake does not decrease the rise in plasma corticosterone induced by insulin hypoglycemia or swim stress (Fuller and Snoddy, 1977).

The effects of chronic d-fenfluramine on the adrenal hormones are quite different from the acute effects. Basal corticosterone levels are normal and the stress-evoked rise in plasma corticosterone, catecholamines and FFA is substantially decreased (Brindley et al., 1985). These effects were seen with a high drug

dosage (25 mg/kg/day), but not with a lower a dosage (2.5 mg/kg twice daily).

In high concentrations, d-fenfluramine decreases circulating triglycerides (Brindley, 1983; Brindley et al., 1985). This effect may be achieved by a combination of mechanisms including (a) direct inhibition of phosphatidate phosphohydrolase (Brindley, 1983); (b) a long-term decrease in the stress-induced release of corticosterone and adrenal catecholamines that would decrease the total levels of enzyme and (c) increased insulin sensitivity that would oppose the glucocorticoid-induced increase in triglyceride synthesis and release from liver (Brindley et al., 1985).

There is additional indirect evidence that fenfluramine has effects on stress hormones that may be related to its antiobesity actions: (a) administration of d-fenfluramine decreases the ethanol-induced rise in plasma corticosterone (Brindley et al., 1979); (b) d,l-fenfluramine is effective in reversing obesity resulting from overeating induced by either tail pinch (Antelman et al., 1979) or central administration of muscimol (Borsini et al., 1982) both of which are proposed animal models of stress-induced eating; (c) glucocorticoid-induced obesity in humans is responsive to d,l-fenfluramine treatment (Cameron et al., 1972; Tomlinson et al., 1975) and (d) d,l-fenfluramine may be effective in treating obesity associated with stress-induced eating (Antelman and Caggiula, 1979; Robinson et al., 1985).

Acutely, d-fenfluramine stimulates glucocorticoid release; however, chronic glucocorticoid stimulation does not appear to be consistent with the drug's effects on glucose metabolism and weight loss. Brindley et al. (1985) reported that chronically administered d-fenfluramine decreased the stress-induced peak in corticosterone, but only at a very high dose (25 mg/kg) and not at lower doses. The stressor used in this study was administration of a fructose load and this metabolic stimulus may be affected by fenfluramine in ways that might not be generalizable to other types of stressors.

At high concentrations, d,l-fenfluramine causes an acute increase in plasma FFA and glycerol and a decrease in plasma triglycerides (Pawan, 1970) indicating that lipolysis is stimulated. This is also consistent with recent reports of prolonged stimulation of lipolysis by d-fenfluramine (Nicolaidis and Even, 1986). The effects of chronic administration of lower doses is not known. The increased insulin sensitivity and possible inhibition of glucocorticoid release observed with chronic fenfluramine might tend to slow lipolysis.

Animal Models of Obesity

The evidence that fenfluramine is more effective in obese than in lean animals has been presented briefly. If this is true, previous data obtained from normal weight laboratory animals may not be directly applicable to obese humans. Two animal models of

obesity were used in the present experiments: diet-induced obesity produced by feeding a varied, palatable diet (DIO) and obesity developed subsequent to ovariectomy (OVX).

In preliminary studies, female rats maintained on a diet of laboratory Chow supplemented with sweetened condensed milk and chocolate chip cookies gained weight at a much greater rate than Chow-fed controls and after 3 months, weighed an average of approximately 100 g (25%) more than controls. Others have found that weight gain on similar diets is variable with regard to species, sex and age (Hill et al., 1983; Levin et al., 1987).

Feeding of a highly palatable diet induces obesity in laboratory animals. This model of diet-induced obesity (DIO) seems to be most relevant to many of cases of human obesity. This is not to deny the importance of heredity and individual differences in metabolism; however, palatability and food composition are extremely important determinants of food intake and availability of a variety of highly palatable foods apparently can override early satiety signals. This model also may be relevant in some regards to special cases of overeating such as Bulimia Nervosa, which is characterized by ingestion of large amounts of palatable (high carbohydrate, high fat) foods.

Females of many species, including humans and rats, have a higher percentage of body fat, the degree and disposition of which is affected by ovarian hormones (Wade and Gray, 1979). Females

also have a greater ability to conserve energy stores and alter food efficiency. Naturally occurring or experimentally induced fluctuations in the levels of circulating gonadal hormones produce changes in body weight and fat content, as well as relevant behaviors such as food intake and voluntary exercise (Wade and Gray, 1979). Ovariectomy rapidly and reliably produces weight gain and obesity in rats that can be reversed by administration of physiological levels of estrogen.

Features common to these and other animal obesity models and possible causes of obesity include (a) energy imbalance due to increased food intake, decreased metabolic rate, decreased thermogenesis or increased efficiency of metabolism; (b) increased adiposity due to hyperplasia and/or hypertrophy of adipose tissue; (c) hyperinsulinemia and insulin resistance; (d) enhanced lipid accumulation due to increased lipogenesis and/or decreased lipolysis and (e) hyperlipidemia.

A part of the variability in studies on diet-induced obesity may be due to differential changes in size and number of adipocytes with different ages and lengths of exposure to special diets. This is an important issue with regard to both white and brown adipose cellularity. With short-term feeding of a cafeteria diet, interscapular brown adipocytes (IBAT) increase in size (Tulp, 1981). With relatively long-term feeding of a similar diet, cell size may decrease, but cell number increases (Triscari et al., 1985; Tulp, 1981).

Similar changes may occur in white adipose tissue (WAT). Removal of a palatable diet from obese rats can result in a temporary increase in thermogenic capacity (Levin et al., 1983). These animals may then become leaner (Stephens, 1980) and show resistance to future development of obesity (Brooks et al., 1981). After 9 weeks or more of overfeeding, hyperplasia may begin to develop (Faust et al., 1978; Obst et al., 1981) and animals may subsequently maintain relatively higher body weights (Rolls et al., 1980). Metabolic activity of adipocytes may change as cells change size when reported as activity per cell (Brunzell, 1979), or as a function of surface area (Brunzell and Greenwood, 1983).

Blundell and Hill (1985) have found a difference in the effects of d-fenfluramine administered in drinking water on food intake and weight loss of animals on a cafeteria diet depending on the length of time on the diet. During the dynamic phase of weight gain, d-fenfluramine was equally effective in cafeteria- and Chow-fed animals; however, after 76 days on the cafeteria diet, weight gain had reached a plateau and d-fenfluramine was significantly more effective in obese animals than in Chow-fed controls.

The time course for development of adipocyte hyperplasia appears to be of critical importance in assessing the long-term effects of overfeeding and weight changes subsequent to changes in diet. Overfeeding for a period of time that is sufficient to

allow hyperplasia to develop appears to have long-lasting or permanent effects on body weight. In humans or experimental animals with increased adipocyte number, dietary restriction or anorectic drug treatment may decrease the amount of lipid stored in existing adipocytes; however, when storage is reduced to a given level, further reductions become increasingly difficult. When dietary restriction is eased, or drug treatment terminated, adipocytes will not have decreased in number and may simply refill.

Enzymes of Energy Utilization and Storage

Dietary Obesity and Thermogenesis.

Experimental evidence (eg. Rothwell and Stock, 1979a, 1979b), along with clinically observed variations in efficiency of metabolism, suggest that increased caloric intake is accompanied by increased energy expenditure and an increase in diet-induced thermogenesis (Danforth, 1981; Landsberg and Young, 1981; Rothwell and Stock, 1979b). In experimental animals, this has been attributed to increased metabolic activity in brown adipose tissue (BAT) (Rothwell and Stock, 1979a; Stirling and Stock, 1960). Cafeteria feeding for a period of 2 weeks has been associated with hypertrophy of interscapular brown adipose tissue (IBAT) (Armitage et al., 1983; Himms-Hagen et al., 1981; Rothwell and Stock, 1979b) and unmasking of GDP-binding sites in BAT mitochondria (Himms-Hagen et al., 1981). Analogous changes are seen with cold exposure (Armitage et al., 1983) and with chronic administration

of NE (Desautels and Himms-Hagen, 1979; Himms-Hagen et al., 1981). The increase in IBAT weight induced by diet persists with return to Chow feeding (Tulp, 1981) whereas IBAT size returns to normal upon termination of cold exposure (Himms-Hagen et al., 1972).

Sympathetic activity is critical in the regulation of thermogenic activity in BAT and other tissues (Depocas et al., 1978; Desautels and Himms-Hagen, 1979; Fain et al., 1973; Foster and Frydman, 1978; Horowitz, 1973; Seydoux et al., 1977). In both brown and white adipose tissue, sympathetic activity at beta-adrenergic receptors stimulates lipolysis providing FFA for subsequent beta-oxidation. In BAT, FFA may also uncouple mitochondrial oxidative phosphorylation (Fain et al., 1973). Increased NE turnover reported in cafeteria-fed rats may be a mechanism by which thermogenesis is stimulated (Levin et al., 1983).

Swann (1984a) reported diet-induced changes in activity of the enzyme ($\text{Na}^+ - \text{K}^+$)ATPase in BAT and muscle that appeared to be regulated by beta-adrenergic receptors. Cafeteria feeding increased ATPase activity and this activity remained elevated with return to regular diet. The animals in that experiment increased their caloric intake by 80% but did not gain weight relative to controls. Food deprivation resulted in decreased ATPase activity that persisted upon refeeding. During the period of refeeding, those animals gained weight approximately 3 times faster than

nondeprived controls. Insofar as ATPase activity is an indicator of thermogenic activity in BAT, that experiment suggests that overfeeding associated with increased activity in BAT does not produce weight gain, but food restriction produces decreases in BAT activity that may increase the efficiency of subsequent weight gain.

Acceptance of the notion that BAT thermogenesis is crucial to changes in energy metabolism with variation in diet is not universal. In carefully controlled metabolic studies, Armitage et al. (1983) were able to account for increased energy expenditure during cafeteria feeding entirely by adding the increased energy costs of digestion, fat synthesis and increased body size. Triscari et al. (1985) reported hyperinsulinemia and greatly increased efficiency of energy utilization in rats with diet-induced obesity. Removal of IBAT or section of the sympathetic fibers innervating this tissue, increases the deposition of lipids in white adipose tissue during sucrose feeding (Granneman and Campbell, 1984). Similar results have been interpreted as evidence in favor of an "energy burning" role for BAT; however, in the experiments conducted by Granneman and Campbell, *in vivo* lipogenesis in BAT was also stimulated. Denervation decreased this response by 75%. As much as 91% of variations in IBAT weight may be accounted for by variations in body fat (Hervey and Tobin, 1983). Thus, BAT may serve as a lipid storage reservoir, removal of which necessitates increased storage in white adipose tissue.

Hill et al. (1983) were able to divide rats into two groups on the basis of their response to a high fat diet: one group showed increased efficiency of energy utilization, no change in diet-induced thermogenesis and positive weight gain and a second group of rats of similar age, sex and strain showed an increase in meal-associated thermogenesis but no change in efficiency of utilization and did not gain a significant amount of weight. Those authors found no correlation between diet-induced thermogenesis and oxygen consumption of IBAT.

Perhaps some of the disparity in the results and interpretations of the experiments discussed above can be explained with careful attention to time course, diet composition and individual or species variability. Rats fed a cafeteria diet for 3-4 weeks gain excess body weight in spite of increased thermogenesis in some, but not all, cases (Rothwell and Stock, 1979b). Levin et al. (1983) found increased NE turnover in BAT of rats fed a cafeteria diet for 7 days but, after 3 months on that diet, plasma NE levels were lower than Chow-fed controls and NE turnover in organs was decreased. In that study, NE-stimulated lipolysis was decreased in BAT and cold-induced thermogenesis was impaired, both of which suggest defects in sympathetic postsynaptic receptors.

Reports on the effects of fenfluramine on BAT activity and thermogenesis are conflicting. Lupien and Bray (1985) have reported increased GDP-binding in vivo with high dosages of

d,l-fenfluramine (20 mg/kg) but not with lower dosages or with the high dosage in vitro. In those experiments, GDP-binding remained elevated with chronic (11 days) drug treatment and those authors claimed that food intake had returned to normal within that time. We have observed that with dosages of d,l-fenfluramine as high as the one used by Lupien and Bray, complete anorectic tolerance usually does not develop, at least within 2 weeks, and in the Lupien and Bray experiment, food intake of d,l-fenfluramine-treated rats appeared to be comparable to controls only on the last day (day 11); that is, intakes appeared to be significantly lower up until day 10.

Rothwell et al. (1982) found no effect of d,l-fenfluramine on NE uptake or $(\text{Na}^+-\text{K}^+)\text{ATPase}$ activity in BAT; however, basal ATPase appears to be regulated by alpha-1-noradrenergic receptors whereas the reported diet-induced increases require beta-noradrenergic receptor activation (Swann, 1984a). Beta-receptor activity may be sensitive to modification by corticosterone and gonadal steroid hormones as well as concentration of NE and other factors that may be affected by fenfluramine and by diet. Furthermore, the obese state may be associated with changes in fluidity of a number of membranes including those of adipocytes (York et al., 1982) that may, in turn, reduce coupling of beta-receptors and adenylate cyclase and decrease stimulated $(\text{Na}^+-\text{K}^+)\text{ATPase}$ activity.

Even and Nicolaidis (1986) have reported that acutely administered d-fenfluramine decreases the efficiency of locomotor activity in rats but has no effect on basal metabolism. Rowland (1986) found no difference in the effect of d,l-fenfluramine on body weights of exercising versus sedentary hamsters. If fenfluramine has subtle effects on metabolism, it may be that these effects will be more apparent in animals with higher ratios of fat/lean tissue and such a difference may help explain the increased efficacy of fenfluramine in obese animals and in females.

The activity of $(\text{Na}^+-\text{K}^+)\text{ATPase}$ is an index of energy utilization and cellular activity. Activity of the ATPase enzyme in BAT, which may reflect changes in thermogenesis, has been shown to be sensitive to changes in diet (Swann, 1984b). Enzyme activity in BAT may also be responsive to ovarian hormones (Bartness and Wade, 1984). Swann (1984a) reported increased ATPase associated with overfeeding in the absence of weight gain and in similar experiments, fasting resulted in decreased enzyme activity and enhanced fuel efficiency.

Hormonal effects on ATPase have not been studied extensively. Adrenal hormones alter ATPase activity in some tissues (Charney and Donowitz, 1976) and may have an inductive effect, at least in developing animals (Huttenlocher and Amemiya, 1978). Estrogen stimulates (Knudsen, 1976) and progestins inhibit

(LaBella et al., 1979; Yamamoto, 1978) ATPase activity in the anterior pituitary of ovariectomized rats. In vitro, insulin directly stimulates ATPase in some muscles under conditions of less than maximal activity (Gavryck et al., 1975; Clausen and Kohn, 1977).

Changes in thermogenesis are accompanied by changes in body temperature or in heat exchange. Levin et al. (1983) found that rats with diet-induced obesity showed impaired thermogenic responses when exposed to cold. Reports on the effects of fenfluramine on body temperature are variable, but generally slight decreases have been reported. Pawlowski et al. (1980) and Sugrue (1981, 1984) reported decreases of 1-2 °C in rectal temperatures of d,1-fenfluramine-treated rats. This effect appeared to be 5-HT dependent. It is possible that the decreases in temperature are due to increased heat loss since 5-HT is a peripheral vasodilator.

Regulation of Lipid Storage and Utilization

There are two triglyceride lipases in adipose tissue that are the primary regulators of storage and mobilization of lipids. Hormone-sensitive lipase (HSL), the rate limiting enzyme in the breakdown of adipocyte triglycerides to release FFA and glycerol is critically responsive to plasma catecholamines. Lipoprotein lipase (LPL) catalyzes the breakdown of triglycerides from plasma sources providing FFA for reesterification and storage within the adipocyte and is the rate limiting enzyme in the formation of

triglycerides in adipocytes (Desai and Hollenberg, 1975; Garfinkel et al., 1967). This enzyme is also found in other tissues such as cardiac and skeletal muscle, where it catalyzes the hydrolysis of triglycerides to provide FFA for fuel.

Fenfluramine may have effects on HSL at the level of enzyme activation: d,l-fenfluramine inhibits lipolysis stimulated by NE, dibutyryl-cAMP, caffeine, ACTH and glucocorticoids (Dannenbury and Kardian, 1969; 1970).

The activity of LPL is affected by insulin levels; however, there is debate over the exact role and importance of insulin in regulation of LPL and over its relative importance in human obesity. Some studies suggest that plasma insulin levels are the major regulator of LPL activity (Cryer et al., 1976); however, other data suggest that the role of insulin on LPL activity is a permissive one (Eckel et al., 1978; Turkenkopf et al., 1982). In any event, insulin levels in vivo are well correlated with LPL activity and in vitro, LPL activity can be varied by changing insulin levels in the medium (Cryer et al., 1976). Furthermore, fat cell size which is regulated by the activity of LPL and HSL is an important determinant of insulin resistance which in turn, affects insulin secretion.

The ovarian hormones have important effects on LPL activity that may mediate their effects on food intake and body weight (Wade and Gray, 1978). Estradiol also appears to have important

effects on BAT and thermogenesis (Kemnitz et al., 1983) that are influenced by sympathetic activity in this tissue (Bartness and Wade, 1984). High affinity cytoplasmic binding sites have been found for [3 H]estradiol (Wade and Gray, 1978) and a synthetic progestin ([3 H]R5020) (Gray and Wade, 1979) in adipose tissue. Increases in LPL activity following ovariectomy precede changes in feeding behavior by about 12 hours (Steingrimsdottir et al., 1980). The increase in LPL activity following ovariectomy is prevented or reversed by administration physiological doses of estradiol (Gray and Wade, 1981). Gonadal steroids may also affect lipid metabolism by producing changes in HSL activity. Exogenously administered estrogen potentiates catecholamine-stimulated lipolysis (Benoit et al., 1982; Hansen et al., 1980). Thus, the gonadal steroids may affect lipid storage in adipose tissue by producing reciprocal shifts in LPL and HSL activity.

Lipoprotein lipase is particularly sensitive to changes in diet and body weight. Adipocyte LPL is related to cell size (Hietanen and Greenwood, 1977; Brunzell, 1979) and is elevated in several animal models of obesity as well as in a population of obese and formerly obese humans. The increase in LPL activity may occur in pre-obese animals prior to hyperphagia (Boulangue et al., 1979) and development of hyperinsulinemia (Turkenkopf et al., 1982). Obese humans have increased LPL activity per adipocyte, but no difference in activity per gram tissue (Guy-Grand and

Bigorie, 1975; Lithell and Boberg, 1978; Pykalisto et al., 1975; Taskinen and Nikkila, 1977). Whether or not this indicates a functional increase in activity is not clear.

The rate of weight loss in dieting humans is inversely correlated with progressive changes in LPL activity. Thus during the initial rapid weight loss that accompanies a hypocaloric diet, there is no change in LPL activity; however, with continued caloric restriction, LPL activity is increased, weight loss slows and the individual often reports increasing discomfort with the restricted diet (Schwartz and Brunzell, 1981).

Formerly obese humans with stable, reduced weights have adipocyte LPL activity increases of 3-4-fold compared to activity measured before weight loss. With return to the original obese weight, LPL activity is restored to former levels (Schwartz and Brunzell, 1981). These changes in LPL activity associated with changes in body weight may have important implications for understanding the difficulty in sustaining weight loss and preventing regain.

Brain Reward Systems and Electrical Brain Stimulation (ESB)

Electrical current delivered via electrodes implanted in the area of the medial forebrain bundle of the lateral hypothalamus will produce both consumatory behaviors and reinforcing effects. Animals readily learn to press a bar in order to deliver intracranial stimulation (ICSS). When food is present, electrical

stimulation to this area will induce consumatory behaviors including eating and gnawing.

Theories concerning electrical stimulation of the brain (ESB) include drive-reduction, incentive-motivation and reward. In accordance with drive reduction theories, when a stimulus elicits a consumatory response, that response ordinarily persists until the stimulus is removed and satiation occurs. In a hungry animal, exteroceptive and interoceptive food stimuli are rewarding. As the hunger is diminished, the same stimuli become less rewarding or aversive. Thus ICSS can be described as a consumatory behavior that does not satiate. The reinforcing effects of ICSS have also been attributed to an appetitive-motivational system. Stimulation produces a central state that induces the animal to obtain reinforcement by appropriate motor responses. Olds (1976) conceptualized the central nervous system as a reward and learning machine with motivation, reward and learning the key words in the programming of behaviors. Subjects learn to do things if they are rewarded for doing so and if they are motivated. Behavior is steered and eventually terminated by rewards.

Two approaches have been used to study the interrelationship of ESB and motivation. One is to vary motivation (e.g. starvation or overfeeding) and look at ICSS behavior and the other is to deliver ESB and examine the effects on motivated behaviors.

Responding rates for ICSS are greatest when stimulation electrodes are located in the lateral hypothalamus, but maps of "reward" sites extend from the olfactory paleocortical area of the telencephalon spreading across the floor of the forebrain and mid-brain and along a path near the roof of the medulla.

Rates of ICSS follow a circadian rhythm with the highest rates occurring at night and peaking in the final hours of the dark cycle. The ICSS rhythm matches rhythms in food and water intake and in body temperature. When sweetened milk is provided as food, the peak ingestion is earlier and the variability in the pattern is increased (Terman and Terman, 1976).

In the lateral hypothalamus, ICSS responding is rapid and animals show signs of marked sympathetic nervous system arousal: tachycardia; hyperthermia; hypertension and general stress responses including elevation in plasma glucocorticoids and catecholamines.

Electrical stimulation delivered to sites that support ICSS elicits consumatory behaviors, the nature of which are dependent upon the location of the electrode as well as the availability of goal objects. Eating and drinking in response to ESB are not like those behaviors in hungry or thirsty animals, but appear stereotypical. Rather than eliciting natural-like motivated behaviors, ESB may evoke well-practiced response patterns. Thus ESB at certain sites may evoke or sensitize a motor response that tends to

channel behavior in a given direction. These are fragmentary responses that do not duplicate natural motivational states and may be programmed in the brain stem (Valenstein, 1976).

Rats made obese by ESB-induced eating decrease their ICSS response rate as a function of weight gain. An animal that increases food intake or ICSS may be responding to either increased positive feedback (appetite) or decreased negative feedback from satiety stimuli. Rolls (1976) located a group of neurons in the lateral hypothalamus that increased their firing rate in hungry animals or in response to naloxone injection in morphine addicted animals and could be "turned off" by food, morphine injection or ESB. Furthermore, single units in the lateral hypothalamus that are activated (either excited or inhibited) by ESB are also activated by natural rewards such as the sight and taste of food but not by eating itself. Thus hypothalamic activation that can motivate an animal to eat will motivate other behaviors as well (Rolls, 1976).

The majority of evidence points toward the involvement of brain catecholamines, especially DA, as mediator of the rewarding effects of ICSS. Amphetamine stimulates ICSS responding and this effect seems to be related to its activity as a DA agonist. Accordingly ICSS responding is increased by dopamine-beta-hydroxylase inhibitors and is decreased by DA, but not (NE, antagonists (Wauquier, 1976). Amphetamine also stimulates other operant

responses, implying that general motor arousal may be responsible; however, the rewarding properties of amphetamine are clearly indicated. Animals will self-administer amphetamine and in humans, it produces euphoria and has a high abuse liability. In a review of pharmacological and anatomical evidence, Wise (1976) concluded that 5-HT is not critical to the rewarding effects of ICSS.

In humans, ESB in the area of the medial forebrain bundle produces sensations of general euphoria, positive mood change, increased talking, pleasurable physical sensations, relief of anxiety and pain reduction. A single session may relieve chronic pain for up to a week (Delgado, 1976). While there is some relationship between electrode placement and the type of sensation reported, this is not consistent either between subjects or in the same subject on different days. Situational factors also have a strong influence.

There are similarities between ESB-induced eating and tail pinch-induced eating. Tail pinch elicits similar behaviors and is thought to be dependent on brain DA (Antelman and Caggiula, 1979). The nigrostriatal DA system is important in sensory and motor function and facilitation of DA increases responsivity to external and internal stimuli (Wise, 1976).

In the laboratory and in nature, there are many examples of stress-induced eating associated with fighting and sexual behavior

(Morley et al., 1983; Antelman and Caggiula, 1979) or other similarly activating conditions. Furthermore, stressful conditions that elicit eating in certain environmental conditions may elicit sexual or aggressive behavior under other circumstances (Morley et al., 1983). Tail pinch-induced eating has been proposed as an animal model of stress-induced eating (Antelman and Caggiula, 1979; Morley et al., 1983). In rats, mild tail pinch reliably produces behaviors similar to naturally occurring stress-related behaviors. Eating, licking and gnawing are especially prevalent. Tail pinch behaviors may be affected by brain 5-HT and brain opioids. Tail pinch behaviors are attenuated by pharmacological manipulations that enhance 5-HT activity in the brain (Antelman and Caggiula, 1979). The opiate antagonist naloxone suppresses tail pinch-induced eating (Morley et al., 1983) whereas the synthetic opioid d-ala-met-enkephalinamide (DAME) stimulates eating (Morley et al., 1983). Acutely, d,l-fenfluramine abolishes tail pinch-induced eating, although tolerance develops to this effect (Antelman and Caggiula, 1979). In contrast, amphetamine anorexia is reversed by tail pinch (Antelman et al., 1979).

Reports of increases in met-5-enkephalin associated with chronic administration of d- or d,l-fenfluramine suggest that this brain opioid may be involved in d,l-fenfluramine anorexia (Groppetti et al., 1984; Dellavedova et al., 1982; Harsing et al., 1982) and tolerance (Groppetti et al., 1984).

Peripheral administration of morphine or icv. injection of met-5-enkephalin or the synthetic analog DAME enhance DA turnover in rat striatum. Morphine and, to a greater extent, DAME stimulate DA release in vivo (Chesselet et al., 1981).

Exogenously administered opiates (e.g. morphine) can stimulate appetite and also ICSS responding. The opiate antagonist naloxone inhibits food intake and decreases ICSS responding. Depletion of 5-HT may stimulate food intake under some conditions and also inhibits ICSS responding. With administration of d,l-fenfluramine, levels of brain opioids gradually increase suggesting that release is inhibited. Opioids may increase the synthesis of 5-HT and administration of morphine releases 5-HT in previously 5-HT depleted animals.

Hoebel et al. (1986) recently have reported that d-fenfluramine decreases ICSS responding in rats with electrodes in an area of the lateral hypothalamus that were also associated with ESB-induced eating and excitation of taste neurons. Those authors concluded that d-fenfluramine inhibits feeding reward and taste reward, and that these effects are mediated by release of 5-HT in the lateral hypothalamus.

CHAPTER III

METHODS

Subjects and Procedures

Subjects

Experiments 1 and 2. One hundred and twenty female Sprague-Dawley rats (initial body weight: mean \pm S.E.M. = 235 ± 2 g) were singly housed in hanging stainless steel cages (10 x 7 x 7 in) in a vivarium that was temperature controlled (23 ± 2 °C). The diurnal cycle was artificially imposed (12 hour light/12 hour dark). Purina laboratory Chow pellets (#5001) and water were available ad libitum unless otherwise stated.

The rats were assigned to one of four groups based on dietary or surgical condition: (1) ad libitum Purina Chow (#5001) (CHOW group); (2) ad libitum cafeteria diet (DIO group); (3) ovariectomized, maintained on Chow (OVX group) and (4) ovariectomized with estradiol benzoate (EB) replacement therapy, maintained on Chow (EB group). These conditions were maintained for 10 weeks prior to the initiation of treatment procedures.

Experiment 3. Female Sprague-Dawley rats were maintained as described in Experiment 1, except that these rats were kept in a room with a reversed lighting cycle (lights on from 16:00 hours until 04:00 hours). This made it convenient for measurements to

be taken during the dark phase when rats are most active. Basal levels of the parameters to be measured are highest at this time. The dietary and surgical conditions were the same as in Experiment 1. Each group initially contained 20 rats.

Experiment 4. Twenty female Sprague-Dawley rats initially weighing 200-300 g were used. The rats were individually housed and maintained under standard laboratory conditions as described in Experiment 1. Purina Chow pellets and water were available ad libitum.

Diet and Surgical Conditions: Experiments 1, 2 and 3

Cafeteria diet. The modified cafeteria diet consisted of a choice of Chow, sweetened milk and chocolate chip cookies. These foods and water were available at all times. The milk was a mixture of 200 g powdered milk and 200 g sucrose per liter of water. This was mixed daily and presented to the rats in 100 ml polypropylene graduated cylinders. The composition of the various foods was as follows. Chow: 3.6 kcal/g; 56% carbohydrate; 23% protein; 5% fat, chocolate chip cookies: 4.7 kcal/g; 70% carbohydrate; 21% fat; 5% protein and sweetened milk: 1.4 kcal/ml; 80% carbohydrate; 20% protein.

Ovariectomies. Bilateral ovariectomies were performed under Equithesin anesthesia (2 mg/kg i.p.). A single midline incision was made in the lower abdomen of the rat and the ovarian horns were located and tied off with cotton suture. Cuts made above

each suture and the ovaries, fallopian tubes and a large section of the ovarian horns were removed. The abdominal muscles were then sewn together and the skin closed with a surgical staple. In rats receiving estradiol benzoate replacement, the steroid (2 ug in 0.1 ml peanut oil) was administered daily by subcutaneous injection beginning on the day after surgery.

Rats were maintained under these conditions for 10 weeks during which time body weights were measured weekly. During the last 2 weeks of this period and throughout the remainder of the experiment, rats in Experiments 1 and 2 received Purina powdered Chow presented in glass jars instead of Chow pellets. This was to facilitate measurement of the food. In Experiments 1 and 2, baseline food intakes were measured during the 4 days prior to the initiation of treatment.

Drug and Dietary Restriction Treatment Procedures: Experiments 1 and 2

Rats within the dietary or surgical condition groups were matched for body weights and assigned to one of five treatment groups: (a) d-fenfluramine treatment (4 weeks); (b) d-fenfluramine treatment (4 weeks) with a 2-week drug-free posttreatment period; (c) dietary restriction (4 weeks); (d) dietary restriction (4 weeks) with a 2-week posttreatment period with an ad libitum feeding schedule and (e) untreated control.

Implantation of osmotic pumps. At the end of the 10-week pretreatment period, all rats were anesthetized with ether and a

small incision was made between their scapulae. In rats from the groups assigned to receive d-fenfluramine, osmotic minipumps (Alzet # 2002) were inserted through the incision and pushed carefully under the skin. These pumps delivered the drug at a rate of 0.5 ul/hour and the drug concentrations were prepared so that each rat received approximately ($\pm 10\%$) 3 mg/kg/day. The skin around the incision was then secured with a surgical staple. Rats that did not receive d-fenfluramine underwent the same procedure except that no pump was implanted. The procedure for each rat was completed within 5 minutes and the rats quickly recovered from the anesthesia. The minipumps used in this experiment delivered drug for 14 days and so this procedure was performed again on day 15 of the 28-day treatment period.

Dietary restriction procedures. Rats in the dietary restriction groups were given access to 75% of their baseline intake of the individual dietary components. The decision to use this percentage was based on preliminary studies in which rats given the same d-fenfluramine dosage by minipump consumed a daily average of 75% of their baseline calories. The intent, therefore was that this group would be approximately pair-fed to the drug-treated groups.

At the end of 4 weeks, rats in the the d-fenfluramine and dietary restriction groups were killed and tissue samples were taken for subsequent examination. Rats in the posttreatment

groups were returned to pretreatment conditions (drug-free, ad libitum food access) for an additional 2-week period. Following this period, these rats and the rats in the untreated control groups were similarly killed.

Surgical Procedures: Experiment 3

Implantation of jugular catheters. During the ninth week of the pretreatment period, jugular catheters were implanted in all rats in Experiment 3. The catheters were made from Silastic medical grade silicone tubing (Dow Corning 602-135: .025 I.D., .047 O.D.) cut to a length of 13 cm and fitted with a curved stainless steel endpiece (approximately 2 cm) made from a 21 gauge needle. A cuff of larger Silastic tubing was used to secure the endpiece in one end of the catheter and another cuff of the same tubing was placed 5 cm from the other end of the catheter. A 2 cm length of Tygon microbore tubing was fitted on the end of the endpiece. The catheters were then sterilized in 95% ethanol and rinsed in 0.9% saline just before they were implanted.

Rats were anesthetized with Equithesin (2 mg/kg, i.p.). A small incision was made in the skin over the right jugular vein just above the clavicle. The vein was then dissected from the connective tissue and tied off near the top of the exposed area using 5-0 silk suture. The vein was held taut by a clamp on this thread and cleaned to expose a small unbifurcated area. A small cut was made in the upper surface of the vein and the catheter

inserted. The catheter was then pushed gently downward through the descending vena cava past the heart and into the ascending vena cava until the tip was in the area of the diaphragm approximately half-way between the heart and liver. The catheter was tested by drawing back blood using a syringe attached to the Tygon cap. The catheter then was secured in the vein by a suture tied near the point of insertion. An incision was made in the scalp of the rat and forceps were run under the skin from the scalp incision to the opening over the jugular vein. The Tygon cap was grasped with the forceps and threaded under the skin to the scalp. The excess tubing was looped under the skin so that only the metal endpiece and Tygon cap were exposed. The skull in the area of the incision was scraped clean and three 1 mm stainless steel surgical screws were placed in the skull in a triangular pattern. The metal endpiece was then attached to the scalp with dental cement and using the screws as an anchor. The skin over the jugular vein and over the scalp was sutured leaving the metal endpiece and Tygon cap the only parts of the catheter exposed. A solution of 8 g polyvinylpyrrolidone (PVP) in 5 ml of heparin solution (1000 units/ml) was infused into the catheter. The volume infused, 0.05 ml, filled the catheter and prevented the clotting of blood in the catheter. The plastic cap was closed by inserting a small (3-4 mm) section of a straight pin that was kept in place except when blood was sampled. Three days later the

catheters were checked by withdrawing blood using a syringe attached to the plastic cap and then filled again with the FVP-heparin solution.

Each group of rats originally contained 20 rats and the groups were to be divided further into d-fenfluramine-treated and untreated control subgroups. The group size was chosen to allow for the possibility that some animals would not survive the catheterization procedure or would die from later complications.

Implantation of osmotic pumps. Six days after the jugular catheters were implanted, the implantation of osmotic minipumps (Alzet #2002, 0.5 ul/hour) began. Each minipump contained d-fenfluramine in a concentration sufficient to deliver 3 mg/kg/day for 2 weeks as described in Experiment 1. Ten rats in each group received d-fenfluramine and the remaining rats in each group were implanted with empty pumps to serve as controls. The pumps were implanted in 3 squads of rats at 2-day intervals so that testing could be performed on 24 rats on 2 consecutive days. Each set of tests involved a 2-day series of procedures so drug treatment began in 24 rats; 2 days later treatment began in a second group followed by the third group 2 days after that. For the purpose of testing, the rats were divided into three groups of 24 containing 3 rats from each of the dietary or surgical and treatment conditions. Following the 2 weeks of d-fenfluramine administration, there was a 2-week drug-free period.

Surgical Procedures: Experiment 4

Rats were anesthetized with Equithesin (2 mg/kg) and were placed in a stereotaxic holder (Kopf) with their skulls level to the plane of the ground. The skull was exposed, scraped clean and three stainless steel 1 mm surgical screws were placed in the skull in a triangular pattern. A small hole was drilled in the skull and a stainless steel bipolar electrode was implanted in the region of the lateral hypothalamus (the electrode was lowered 7.5 mm from the dura at coordinates 4.6 mm posterior and 1.4 mm lateral to bregma). The cap of the electrode was then attached to the skull with dental cement and using the screws as an anchor. When this was dry, the rat was removed from the holder and returned to its cage. All rats were allowed to recover from the surgery for at least 5 days before behavioral training was begun.

Food and Body Weight Measurements: Experiments 1 and 2

During the baseline and subsequent treatment periods, intakes of Chow and cookies were measured every 2 days by weighing the food, and each 2 days, the food was replaced with fresh. Milk was measured and refreshed daily. The amount of milk consumed was read from the calibration lines on the graduated cylinders. Intakes of each food were converted to kilocalories. Total intakes were expressed as a percentage of baseline for each 2-day measurement. Intakes of the different foods in the DIO groups were analyzed also.

Body weights were measured weekly throughout the experiment. Weight changes during the three phases of pretreatment, treatment and posttreatment were analyzed. Food efficiency was calculated from the equation: [weight change (grams)/kilocalorie ingested].

Tissue Dissections: Experiments 1 and 2

At the end of the treatment interval, all rats except those in the "recovery" groups and the untreated control groups were killed by decapitation. Food was removed at 20:00 hours on the final day of the treatment period and the following procedures were begun at 08:00 hours the next day. Two weeks later, similar procedures were carried out on the remaining animals.

Trunk blood was drained into polypropylene tubes and stored on ice. Blood samples were centrifuged at 760 x g for 20 minutes and the plasma was removed and stored in polypropylene tubes at -80 °C.

Tissues samples from brain (telencephalon and hypothalamus), gastrocnemius muscle, interscapular brown adipose tissue (IBAT) and subcutaneous (retroperitoneal) and abdominal (inguinal) white adipose tissue (WAT) were rapidly dissected on ice, stored on dry ice until the procedure was completed and then stored at -80 °C until assays were performed. Dissections were performed by three workers. As each rat was decapitated, one worker removed brain tissue, another adipose tissue and the third, muscle.

Brain dissections. The brain was removed from the skull and cut sagittally along the midline. One half was frozen intact. From the other half, the telencephalon was removed by cutting posterior to the striatum and then peeling back the cortex. The hypothalamus was lifted from the base of the brain with small forceps.

Adipose and muscle tissue dissections. Brown adipose tissue was exposed by cutting the skin over the interscapular region and identified by its color and location. It was then dissected from the surrounding muscle, cleaned of any WAT or muscle and divided into three pieces, two of which were stored dry ice, while the third was put in a glass vial containing 10% formalin-saline solution. Retroperitoneal WAT was obtained from above the kidneys and inguinal WAT was obtained from the area surrounding the ovaries (this region was inferred in ovariectomized rats from the location that the ovaries would have been). Two samples of each tissue were frozen and an additional sample of inguinal tissue was stored in formalin solution. The gastrocnemius muscle on each leg was dissected, cut near its origin and insertion and frozen.

Experiment 3: Procedures

Collection of plasma samples. Plasma samples were collected under standard conditions between 14:00 and 16:00 hours on the third day of the treatment interval. Food was removed at 10:30 hours on the test day. Water was available during this period.

The rats were held in a loosely wrapped towel while approximately 0.5 cc of blood was withdrawn and replaced with an equivalent amount of 0.9% saline. The blood was put into polypropylene microcentrifuge tubes containing approximately 5 ul of 4% heparin-sodium fluoride solution.

Measurement of colonic temperatures. Following the collection of plasma samples, body temperatures were measured using a colonic thermistor (YSI 400 Scientific Instruments). The probe was inserted approximately 4 cm into the rectum and held in place until a stable reading was obtained. The rats were then returned to their cages.

Glucose tolerance tests. The collection of plasma samples and measurement of colonic temperatures took approximately 2-3 minutes/rat. When those procedures were completed, glucose tolerance tests began. A 25% glucose solution (0.2 cc/kg) was infused slowly over a 5-8 second period via the jugular catheter. The catheter was then rinsed by infusing 0.1 cc 0.9% saline and the rats were returned to their cages. Exactly 20 minutes later, blood samples were taken again. The first 0.05 cc of blood was discarded to prevent contamination of the sample by glucose that may have remained in the catheter even though it was rinsed. A 0.15 cc sample was then withdrawn and placed in a microcentrifuge tube containing 5 ul of the heparin-NaF solution. The catheters were filled with 0.05 cc FVP-heparin and

closed. The rats were returned to their cages and when the entire procedure was completed, food was returned. Blood samples were centrifuged at 2000 rpm for 20 minutes. The plasma was then removed and stored in polypropylene tubes at -80°C .

On the fourth day of d-fenfluramine treatment, rats were placed in a cold room (4°C) for 4 hours (from 10:30 until 14:30 hours). The rats were kept in their home cages and the cage rack containing 24 rats was rolled into a walk-in refrigerated room. Food was removed at 10:30 hours as before. At 14:30 hours, blood samples were collected and body temperatures were measured as described above. Glucose tolerance was not tested on this day. When the procedure was completed for all the rats, they were returned to the colony and given food. Blood samples were processed as before.

Experiment 4: Procedures

Training and testing was conducted in 12 x 12 x 15 in chambers equipped with a lever that activated a constant current stimulator and event counter. Most rats readily learned to press a bar to receive ICSS by simple behavioral shaping procedures. Rats were trained to bar press at the lowest stimulation level that produced essentially constant responding. Daily training and testing sessions were 1 hour/day in the later part of the light cycle (15:00-17:00 hours). When the rats were responding reliably, baseline measurements were taken. The number of responses was recorded at the end of each 15 minute interval.

Following 3 days of baseline measurements, drug treatment began. Rats were tested with increasing dosages of d- and d,l-fenfluramine. Each dosage was administered for a 3-day period with a drug-free interval of at least 1 day between different dosages and a 3-day drug-free period at the end of the experiment. Drug dosages were 2, 4 and 8 mg/kg of both compounds. Each rat received both drugs; half received the series of d-fenfluramine dosages first and half received the racemate first.

When ICSS testing was completed, the rats were screened for "stimulation bound" eating or gnawing by placing them in a stimulation chamber with food, but no bar, present. Electrical stimulation matched to a level at which the rats had responded for ICSS, was delivered at a rate of 5 pulses/second for 30 seconds intervals (30 seconds on/30 seconds off). If the rats did not respond to this stimulation, the voltage was gradually increased to see if a response could be evoked. The rats were judged positive or negative for the response.

Following the termination of the study, the rats were given an overdose of pentobarbital anesthesia and perfused with 10% formalin solution. The brains were removed and stored in formalin solution, and later were cut on a freezing microtome at a thickness of 30 μ m and stained with methylene blue. Electrode placement was verified by microscopic examination of the brain sections.

Measurement of Adipocyte Diameter: Experiments 1 and 2

Estimates of adipocyte diameter were determined by the method of Gurr and Kirtland (1978). Interscapular BAT and inguinal WAT were dissected and stored in 10% formalin solution as described above. The tissues were cut 3-4 weeks later on a freezing microtome at a thickness of 150 μm for WAT and 50 μm for BAT. The 150 μm thickness is optimal for viewing WAT but it is necessary to cut thinner sections of BAT in order to allow enough light to pass through the denser tissue to enable viewing with a light microscope. Sections were mounted using 0.9% saline and coverslips were placed over the tissue. Slides were immediately viewed under a Nikon M-35FA Fluophot light microscope at a magnification of 6.5x and photographed using high contrast black and white film (Kodak Pan X). Cell diameters were determined from the photomicrographs by comparison with a micrometer photographed at the same magnification. In BAT sections, cell diameters were measured on a horizontal line and a vertical line across the photograph and the mean diameter computed for each sample. In WAT sections every intact cell in the photograph was measured at its widest point.

Measurements of Brain Monoamines: Experiments 1 and 2

Telencephalic and hypothalamic content of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and DOPAC were measured by high pressure liquid chromatography with electrochemical detection (HPLC-ED). Tissues were weighed to the

nearest milligram and homogenized in 5 vol of 0.2 N cold perchloric acid containing 2.5×10^{-5} M EDTA and 10^{-6} M MOPET as an internal standard. The homogenates were centrifuged at $10,000 \times g$ for 10 minutes in a Sorvall RC-5B refrigerated centrifuge at a temperature of 0 ± 5 °C. The supernatant was decanted and small aliquots were stored in sealed glass vials at -80 °C. Analyses were performed within 90 days.

The chromatographic system consisted of a Waters M-45 pump, a uBondpak C-18 reversed-phase column and a Bioanalytical Systems model LC-4B electrochemical detector with a glassy carbon electrode. An applied potential of +0.7 volts was set against an Ag/AgCl reference electrode. Samples were kept frozen until just prior to time they were injected onto the column.

Peak areas were computed by a Perkin-Elmer Sigma 15 Chromatography Data Station. Sample concentrations were calculated by comparing peak areas of samples to areas of standards prepared at a concentration of 10^{-6} M and corrected for variation in chromatographic conditions by comparing the peak area of the internal standard in samples and in a standard peak.

The mobile phase for catecholamine detection consisted of 84 parts 0.1 M NaH_2PO_4 and 16 parts methanol with 2.6×10^{-3} M octyl sodium sulfate, 1.0×10^{-4} M EDTA and 2.5×10^{-4} Et_3N at a pH of 3.4 (Wagner et al., 1982). The mobile phase for detection of indoleamines consisted of 0.1 M citric acid and 0.1 M sodium

acetate in 20% methanol solution. Monoamine concentrations were expressed as nanomoles/gram wet tissue. Estimates of monoamine turnover were determined from the ratios of 5-HIAA/5-HT and DOPAC/DA.

Biochemical Assays

Measurement of $(\text{Na}^+-\text{K}^+)\text{ATPase}$ Activity: Experiments 1 and 2

Gastrocnemius and BAT tissue samples were stored at $-80\text{ }^\circ\text{C}$ until assays were performed. Tissues were weighed and homogenized in 10 vol of cold 0.25 M sucrose with 5 mM EDTA and 0.1 M sodium deoxycholic acid. The homogenate was centrifuged at $0\pm 5\text{ }^\circ\text{C}$ for 20 minutes at $10,000 \times g$. The supernatant was decanted and aliquoted to clear plastic tubes. Four 100 μl aliquots of each sample were taken for assay of K^+ -p-nitrophenylphosphatase (NPPase) and 10 μl aliquots were taken protein analysis by the method of Lowry et al. (1951).

The activity of K^+ -p-nitrophenylphosphatase (NPPase) is an indicator of $(\text{Na}^+-\text{K}^+)\text{ATPase}$ activity, the measurement of which is relatively insensitive to metabolic competition for ATP and to the endogenous inorganic phosphate pool (Swann, 1984b). Enzyme activity was measured by a modification of the method described by Swann (1984a, 1984b).

The reaction medium consisted of 5 mM MgCl_2 , 10 mM tris (hydroxymethyl)aminomethane (tris)-p-nitrophenylphosphate, 50 mM imidazole (pH 7.5) and 0.6% fatty acid poor bovine serum

albumin. The tissue preparation (100 ul) was incubated with 350 ul of the reaction medium and 50 ul of either 1 mM ouabain or 20 mM KCl in duplicate. Standards were prepared from known concentrations of p-nitrophenol (4×10^{-8} M - 4×10^{-6} M). After incubating at room temperature for 30 minutes, the reaction was stopped by adding 2 ml cold 0.1 M NaOH. The concentration of p-nitrophenol in standards and samples was determined by measuring optical absorption at 410 nm. Ouabain does not inhibit ATPase in the absence of potassium. The estimate of ATPase activity was determined by subtracting the absorbance with ouabain from the KCl reading. Standard values were plotted against their concentrations and sample concentrations were determined from the resulting line of regression. These values were then converted to activity per milligram protein and divided by 30 to give nanomoles/milligram protein/minute.

Measurement of Lipoprotein Lipase Activity: Experiments 1 and 2

The activity of lipoprotein lipase (LPL) was measured by incubating tissues with labeled triglyceride, extracting free fatty acids (FFA) and measuring the radioactivity. This procedure is similar to that of Hietanen and Greenwood (1977) except that tritium-labeled triolein was used instead of the [14 C] isotope.

Retroperitoneal, inguinal and brown adipose tissues were weighed to the nearest milligram and homogenized in cold 0.25 M sucrose containing 1 mM EDTA with the pH adjusted to 7.4 using

KOH. The homogenate was centrifuged at $10,000 \times g$ for 15 minutes at $0 \pm 5^\circ \text{C}$. The lipid supernatant was pushed aside and the post-mitochondrial infranatant removed with a Pastuer pipette. Twenty microliter aliquots of the homogenates were removed for assay of protein content by the method of Lowry et al. (1951). Aliquots of the tissue preparation (100 ul in triplicate) were pipetted into 17 x 100 mm polypropylene tubes, sealed and stored -80°C until enzyme activity was measured. Tissues may be stored in this manner for at least 6 months without affecting measures of enzyme activity (Bartness, 1987, personal communication).

The enzyme substrate was prepared by dissolving 5.4 uCi [^3H]triolein ([9,10- ^3H (N)]triolein, New England Nuclear, 150 Ci/mmol), 36 mg nonlabeled triolein and 1.5 mg lysolecithin in hexane and evaporating the solvent under a stream of air. Three milliliters of serum obtained from donor rats that had been 24-hour fasted and 3 ml 0.2 M Tris HCl (pH 8.0) containing 1% fatty acid poor bovine serum albumin were added to the flask containing the residue. This was emulsified by intermittent sonification at 60 watts (30 seconds on/30 seconds off). The substrate emulsion was then activated by incubating at 37°C for 30 minutes. Tissue aliquots were setup in triplicate in an ice bath. To measure nonspecific activity, one tube in each triplicate received 100 ul of 2 M NaCl. High concentrations of NaCl inhibit enzyme activity.

The reaction was started by adding the substrate emulsion to the homogenates. Samples were incubated for 30 minutes at 37 °C and the reaction was stopped by adding 3.5 ml chloroform-methanol-hexane extraction cocktail (2.3 v: 2.5 v: 1.8 v). To separate the phases, carbonate buffer (6.2 g boric acid and 16.5 g K_2CO_3 /liter) was added in a volume of 1.05 ml to the active tubes and 0.95 ml to the salt tubes. This mixture was vortexed and centrifuged at 760 x g for 20 minutes. One milliliter aliquots of the upper phase were pipetted into glass vials containing 9 ml scintillation fluid and counted for 2 minutes. Total radioactivity was measured by counting 100 ul of the substrate emulsion.

Radioactivity in the upper phase was calculated by subtracting the blank (NaCl tube) from the sample count and dividing by the total radioactivity. This was multiplied by a constant (13.35) that accounts for the efficiency of extraction of FFA (64%), total volume of the upper phase (2.1 ml), starting concentration of triolein (.678 umol/tube), the number of FFA per triolein molecule (3) and the amount of time for which the reaction was measured (0.5 hour). This product is an estimate of the micromoles FFA released/hour/tube. This was converted micromoles FFA/hour/milligram tissue and micromoles FFA/hour/milligram protein.

Measurement of Plasma Insulin Concentrations: Experiments
1, 2 and 3

Plasma insulin levels were assayed by a commercially prepared radioimmunoassay kit (Immuno/nuclear). Plasma samples were diluted and purified by combining 250 ul plasma with 250 ul polyethylene glycol and centrifuging at 760 x g for 20 minutes. Duplicate samples of 200 ul were pipetted from the supernatant into borosilicate tubes. Standards were prepared from rat insulin standard (0-100 uU/ml). Samples and standards were incubated overnight (16-24 hours) with 100 ul Guinea pig anti-insulin serum and 100 ul [125 I]insulin. The following day, unbound radioactivity was precipitated by incubating for 20 minutes at room temperature with 500 ul rabbit anti-Guinea pig precipitating complex. The tubes were centrifuged for 20 minutes at 760 x g and immediately decanted. The tubes were blotted dry and radioactivity was counted in a Beckman gamma counter for 2 minutes.

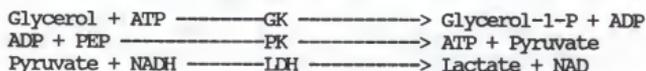
Nonspecific binding (NSB) was determined in the absence of anti-insulin serum. Total binding was the amount of radioactivity in 100 ul [125 I]insulin. The ratio of B/Bo [125 I] was calculated by subtracting the counts per minute (CPM) of the NSB tube from the mean CPM of each sample or standard and dividing by the CPM of the 0 standard minus the NSB and multiplying by 100. Using semi-log paper, a standard curve was drawn plotting B/Bo versus standard concentrations (microunits/milliliter). Sample concentrations were then extrapolated from the linear portion of the curve by logarithmic regression and multiplied by their dilution factor.

Measurement of Plasma Glucose Concentrations: Experiments 1, 2 and 3

Fasted (12-18 hours) plasma glucose was determined for rats in Experiments 1 and 2. An estimate of glucose tolerance was determined from plasma samples taken following the glucose load in Experiment 3. Plasma glucose was measured in a YSI Model 23A automatic glucose analyzer.

Measurement of Plasma Triglyceride Concentrations: Experiments 1, 2 and 3

Plasma triglycerides were measured by the method of Bucolo and David (1973). This method uses enzymatic hydrolysis of triglycerides by *R. delemar* lipase and alpha chymotrypsin. The resulting free glycerol was determined by the following reactions:



The decrease in absorbance of NADH is proportional to the concentration of glycerol.

The triglyceride assay reagent was prepared by combining 5 μM MgSO_4 , 0.9 μM ATP, 0.9 μM phosphoenolpyruvate (PEP), 6 U pyruvate kinase (PK), 2 U lactate dehydrogenase (LDH), 0.4 mg NADH, 400 U lipase from *R. delemar*, 30 U alpha-chymotrypsin and 5 mg bovine serum albumin in 0.1 M potassium phosphate buffer (pH 7.0) to a final volume of 3 ml, the amount of reagent per tube.

To measure triglyceride concentration, the triglyceride reagent (3 ml) and 50 μl of plasma were combined; incubated at

30 °C for 10 minutes and absorption read at 340 nm. The reaction was started by adding 50 ul glycerol kinase solution (2 U enzyme/50 ul phosphate buffer). Tubes were incubated at 30 °C and after 10 minutes a second absorption reading was taken. The second value was subtracted from the first and the difference was multiplied by 883 to obtain milligrams triglyceride/100 milliliters plasma, expressed as triolein.

Measurement of Plasma Corticosterone Concentrations: Experiments 1, 2 and 3

Plasma corticosterone was assayed according to the method described by Gwosdow-Cohen et al. (1982). A 5 ul aliquot of each plasma sample was pipetted into 12 x 75 mm borosilicate tubes in triplicate. Steroids were extracted by mixing with 0.5 ml methylene chloride. The organic phase was then poured into a 13 x 100 mm borosilicate tube and the solvent allowed to evaporate overnight.

The following day, standard tubes were prepared from stock corticosterone solutions (0-1000 ng/ml). Rabbit anticorticosterone antibody was prepared in a 1:2000 dilution in 0.1 M phosphate buffer-saline solution containing 1 M gelatin and 0.1 M sodium azide (PBS-G). This antiserum dilution binds 40% of the [³H]corticosterone added to each tube (Gwosden-Cohen et al., 1982). Tritiated corticosterone with a specific activity of 82.1 Ci/mmol (New England Nuclear) was diluted with PBS-G to yield 13,000 CPM per 100 ul. A dextran-charcoal solution containing

625 mg Norit A charcoal and 62.5 mg Dextran T-70 in 100 ml PBS-G was prepared.

Samples were resuspended in 100 ul PBS-G and 100 ul aliquots of standards were pipetted into test tubes. To all sample and standard tubes 100 ul PBS-G, 300 ul antiserum and 100 ul [^3H]corticosterone were added. Nonspecific binding tubes were prepared in a similar manner with 300 ul PBS-G substituted for the antiserum. Total binding was determined by adding 100 ul [^3H]corticosterone to 400 ul PBS-G. All tubes were then incubated at 4 °C overnight (16 hours). Unbound [^3H]corticosterone was then precipitated by the addition of 200 ul charcoal solution to all tubes except the total binding tube. The tubes were vortexed, placed in an ice bath for 10 minutes and then centrifuged at 760 x g for 5 minutes. A 300 ul aliquot of the supernatant was pipetted into glass vials containing 6 ml scintillation fluid (Scintiverse I). Radioactivity was counted for 4 minutes in a Wallac LB Minibeta scintillation counter.

Efficiency, calculated from nonspecific binding (NSB) divided by total binding was 46-52% for all assays. The ratio of bound/free (B/Bo) [^3H] was calculated by subtracting the NSB counts from the mean CPM of each sample or standard and dividing the difference by the CPM of the 0 standard minus the NSB. A standard curve was drawn on 3 cycle semilog graph paper by plotting B/Bo on the y axis versus standard concentration

(nanograms/milliliter). The linear portion of the curve was then used to extrapolate sample concentrations from their B/Bo [^3H] by logarithmic regression.

Statistics

Experiments 1 and 2

Experiments 1 and 2 were conducted simultaneously. For the purpose of statistical analyses, the data collected on the 120 rats in these two experiments were divided into four groups. In Experiment 1, the effects of 4 weeks of chronic d-fenfluramine administration on food intake, body weight, food efficiency, adipocyte diameter, brain monoamines and peripheral metabolism were compared to untreated control values for rats in each of the four dietary or surgical condition groups: OVX, EB, DIO and CHOW. In the second part of Experiment 1, the effects of d-fenfluramine treatment measured during, or at the termination of the 4-week treatment period were compared to measurements taken after a 2-week drug-free posttreatment period and to untreated control values for rats in the four dietary or surgical condition groups.

In Experiment 2, the effects of 4 weeks of administration of d-fenfluramine were compared to the effects of 4 weeks of dietary restriction in rats from the OVX, DIO and CHOW dietary or surgical condition groups. In the second part of Experiment 2, the effects of 4 weeks of d-fenfluramine treatment or dietary restriction in rats in the DIO and CHOW condition groups were compared to

measurements taken following the 2-week posttreatment period during which drug treatment was terminated and diet-restricted rats were returned to ad libitum feeding.

The results of Experiments 1 and 2 were analyzed by 1-way ANOVA with Newman Kuel's post hoc t-tests or Student's 2-tailed t-tests where appropriate (Ed Sci Apple Software); or by 3-way ANOVA with repeated measures or 2-way ANOVA (ANOVA II Apple Software) with Newman Kuel's calculated by ranking group mean differences and applying the formula: $\bar{x}/\sqrt{MS/n}$. The experimental design for Experiment 1 is shown in Table 1 and the design for Experiment 2 is shown in Table 2.

Experiment 3

The results of Experiment 3 were analyzed by 2-way ANOVA or 3-way ANOVA with repeated measures (ANOVA II). The particular statistics used for each part of Experiment 3 are noted in Chapter IV.

Experiment 4

Data are presented for single animals in Experiment 4 and no statistical tests were performed. Also presented in Experiment 4 are dose-response curves for the effects of d- and d,l-fenfluramine on ICSS responding calculated from groups mean response rates (bar presses/hour) expressed as percentages of baseline response rates.

Table 1. Experiment 1 Design

Treatment	Dietary or Surgical Condition Group			
	OVX	EB	DIO	CHOW
Control (no treatment)	n=7	n=6	n=8	n=8
D-FEN (4wk)	n=7	n=6	n=8	n=8
Control (no treatment)	n=7	n=6	n=8	n=8
D-FEN (4wk)	n=7	n=6	n=8	n=8
D-FEN + 2wk	n=7	n=6	n=7	n=7

Shown are the groups and numbers of rats used in the comparisons made in Experiment 1. (1) The effects of 4 weeks of d-fenfluramine treatment [D-FEN (4 wk)] were compared to untreated control values in the four dietary or surgical condition groups. (2) The effects of 4 weeks of d-fenfluramine treatment measured in rats killed at the end of the treatment period [D-FEN (4 wk)] or following a 2-week drug-free period (D-FEN + 2 wk) were compared to untreated control values. The data for rats in the D-FEN (4 wk) groups and the untreated control groups were used in both parts of Experiment 1.

Table 2. Experiment 2 Design

Treatment	Dietary or Surgical Condition Group		
	OVX	DIO	CHOW
Control (no treatment)	n=7	n=8	n=8
D-FEN (4wk)	n=7	n=8	n=8
75% Diet (4wk)	n=7	n=7	n=7
Control (no treatment)	---	n=8	n=8
D-FEN (4wk)	---	n=8	n=8
75% Diet (4wk)	---	n=7	n=7
D-FEN + 2wk	---	n=7	n=7
75% Diet + 2wk	---	n=7	n=7

Shown are the groups and numbers of rats used in the comparisons made in Experiment 2. (1) The effects of 4 weeks of d-fenfluramine treatment [D-FEN (4 wk)] or dietary restriction [75% Diet (4 wk)] were compared to untreated control values in the OVX, DIO and CHOW dietary or surgical condition groups. (2) The effects of 4 weeks of d-fenfluramine treatment [D-FEN (4 wk)] or dietary restriction [75% Diet (4 wk)] were compared in rats killed at the end of the treatment period; in d-fenfluramine-treated rats killed after a 2-week drug-free period (D-FEN + 2 wk) or diet-restricted rats killed after a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values from rats in the DIO and CHOW condition groups. The data for rats in the D-FEN (4 wk) groups, 75% DIET (4 wk) groups and the untreated control groups were used in both parts of Experiment 2. The data from d-fenfluramine-treated rats [D-FEN (4 wk) and D-FEN + 2 wk] and untreated control data were the same as in Experiment 1.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment 1: Effects of Chronic (4-week) d-Fenfluramine Administration in Lean and Obese Rats

Effects of Ovariectomy (OVX) or Cafeteria Diet (DIO) on Food Intake and Body Weight

The effects of 4 weeks of continuous administration of 3 mg d-fenfluramine/kg/day via osmotic minipump were assessed in female rats from the four dietary or surgical condition groups: ovariectomy (OVX); ovariectomy with estradiol benzoate (EB) replacement (EB); diet-induced obesity (DIO) and Chow-fed, unoperated (CHOW). The effects of this treatment regimen on food intake, body weight and food efficiency were compared to untreated control rats in each the four dietary or surgical condition groups during the 4-week treatment period. At the end of the treatment period, one group of d-fenfluramine-treated rats was killed and tissue samples were collected for measurement of brain monoamines and for assessment of possible peripheral actions of d-fenfluramine. Rats in the untreated control groups, as well as a second group of d-fenfluramine-treated rats, were kept alive for an additional 2-week drug-free period during which measurements of food intake, body weight and food efficiency were continued. At the end of this 2-week period, these rats were killed and tissue samples were collected as before.

The food intake, body weight and food efficiency data presented in this section are based on the following comparisons: (a) the effects of 4 weeks of d-fenfluramine treatment compared to untreated controls in the four dietary or surgical condition groups and (b) the effects of 4 weeks of d-fenfluramine treatment compared to d-fenfluramine treatment followed by a 2-week drug-free posttreatment period and untreated controls in the four dietary or surgical condition groups. Data from the central and peripheral biochemical measurements compared: (a) data from samples taken from d-fenfluramine-treated rats killed at the end of the 4-week treatment period to untreated control rats in the four dietary or surgical condition groups and (b) data from samples taken from d-fenfluramine-treated rats killed at the end of the 4-week treatment period or after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free posttreatment period and untreated controls in the four dietary or surgical condition groups.

Baseline food intake. Baseline food intakes for rats in the four dietary or surgical condition groups: OVX; EB; DIO and CHOW, measured over a period of 4 days during the week preceding the initiation of treatment, are shown in Table 3. Rats in the DIO condition had significantly higher baseline caloric intakes than rats in the other groups. Estradiol-treated (EB) rats had lower caloric intake than the other groups. Rats in the CHOW and OVX

condition groups had similar baseline caloric intakes (1-way ANOVA, $F(3,116) = 67.79$, $p < .001$).

Table 3. Effects of Ovariectomy or Cafeteria Diet on Baseline Food Intakes of Female Sprague-Dawley Rats.

Dietary or Surgical Condition Group	Baseline Intake (x kcal/day)
Ovariectomy (OVX)	89 ± 3 ++
Ovariectomy + Estradiol Benzoate (EB)	74 ± 3 ** ++
Diet-Induced Obesity (DIO)	134 ± 5 **
Chow-Fed, Unoperated (CHOW)	85 ± 2 ++

Shown are group mean ± S.E.M. daily caloric intakes measured during the 4-day baseline period and expressed as mean total kilocalories/day for each of the four dietary or surgical condition groups.

** Different from CHOW group $p < .01$, Newman Kuel's post hoc t-test.

++ Different from DIO group $p < .01$.

Pretreatment body weights. Both ovariectomy and feeding of a varied, palatable diet resulted in marked increases in body weight relative to intact, Chow-fed rats (Figure 1). The two conditions had similar effects on body weight and after 10 weeks, rats in these groups were approximately 25% heavier than rats in the CHOW group. Ovariectomized rats treated with 2 ug estradiol benzoate/day gained less weight than rats in the CHOW condition group during this 10-week period (1-way ANOVA, $F(3,116) = 101.78$, $p < .001$).

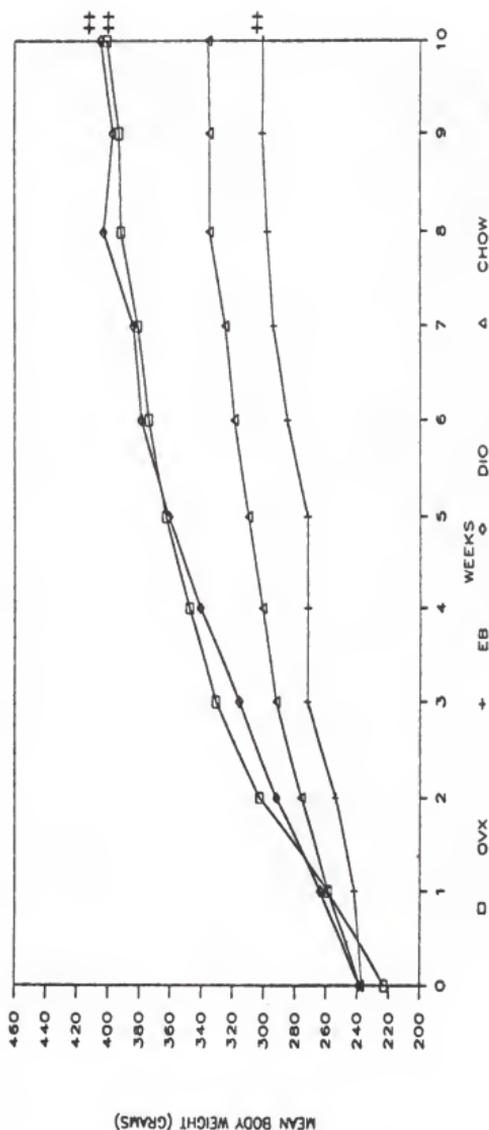


Figure 1. Effects of ovariectomy or feeding of a palatable diet on body weights of female Sprague-Dawley rats. Shown are group mean weekly body weights and rate of weight gain during the 10-week pretreatment period following ovariectomy or introduction of a cafeteria diet. Mean body weights and 10-week cumulative weight gains are shown for rats in the four dietary or surgical condition groups prior to the division into treatment groups.
 ++ Different from CHOW group body weight at 10 weeks, $p < .01$, Newman-Kuel's post hoc t-test.

Effects of d-Fenfluramine on Food Intake, Body Weight and Food Efficiency

Food intake. Food intakes of d-fenfluramine-treated rats and untreated controls from each of the four dietary or surgical conditions were measured at 2-day intervals throughout the 4-week drug treatment period. Mean 2-day caloric intakes were analyzed by 3-way ANOVA with repeated measures design. Total caloric intakes on days 1-2, 7-8, 13-14 and 27-28, converted to kilocalories/day and expressed as percentage of baseline intakes, were incorporated into the statistical analysis. During the 2-week posttreatment period, total caloric intakes on days P1/2, P7/8 and P13/14, expressed as percentage of baseline intake, were analyzed by 3-way ANOVA with repeated measures design. Food intakes on representative days throughout the 4-week treatment period and the 2-week posttreatment period, expressed as percentages of baseline intakes, are shown for rats in the OVX and EB condition groups (Figure 2) and the DIO and CHOW groups (Figure 3).

On the first 2 days of administration, d-fenfluramine treatment significantly suppressed food intakes of rats from the OVX, DIO and CHOW dietary or surgical condition groups compared to untreated control rats in the corresponding condition groups ($F(1,18) = 18.63, p < .005$). The percentages of suppression of food intake on days 1-2 were similar in rats from the OVX, DIO and CHOW groups; however, d-fenfluramine did not have a

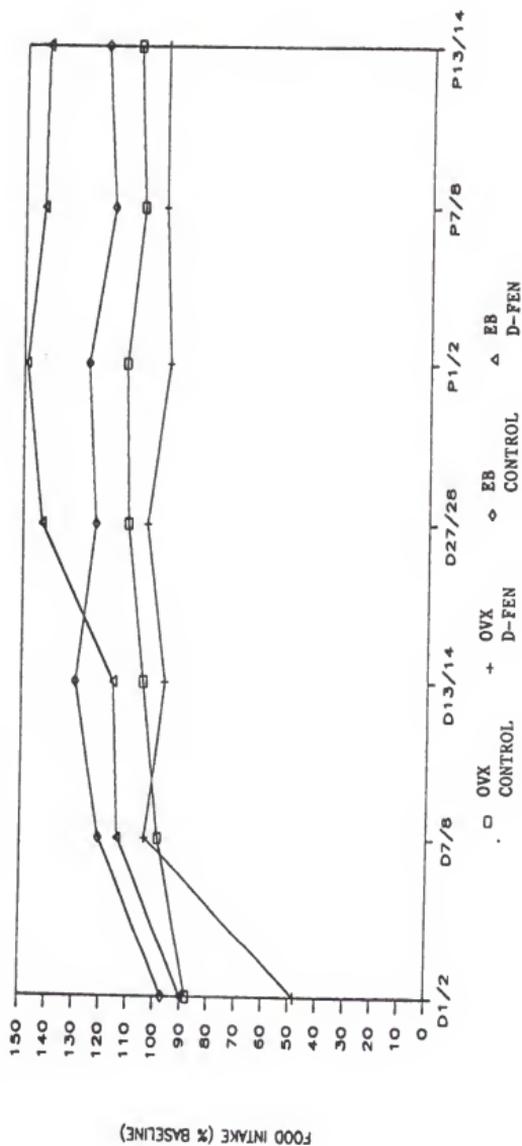


Figure 2. Effects of d-fenfluramine treatment on food intakes of ovariectomized rats (OVX) and ovariectomized rats receiving estradiol replacement (EB). Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN) and untreated controls from the OVX and EB condition groups. Food intakes are expressed as percentages of baseline intakes on representative days during the 4-week treatment period (days D1/2-D27/28) and during the 2-week drug-free posttreatment period (days P1/2-P13/14).

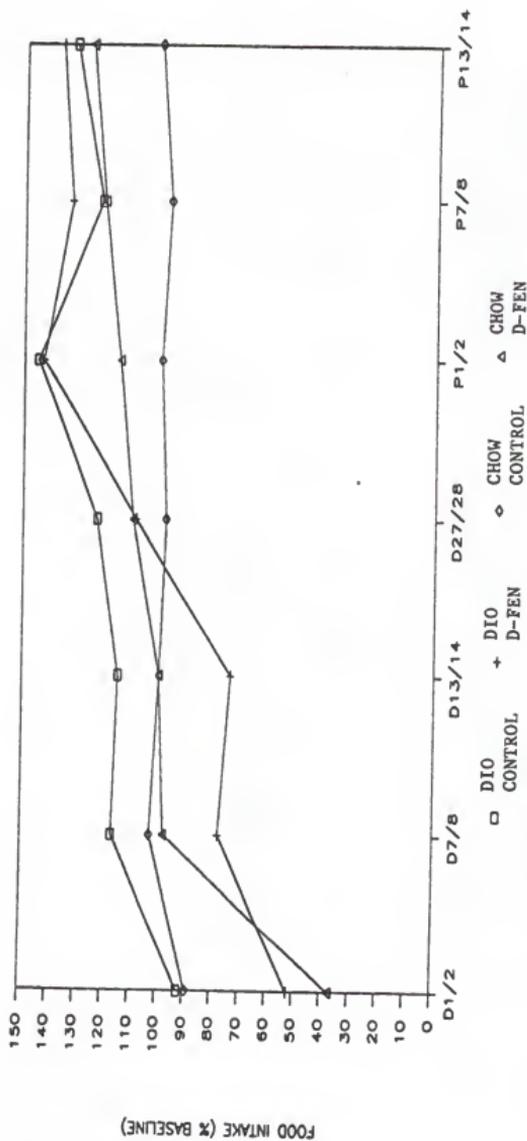


Figure 3. Effects of d-fenfluramine treatment on food intakes of dietary obese rats (DIO) and untreated Chow-fed rats (CHOW). Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN) and untreated controls from the DIO and CHOW condition groups. Food intakes are expressed as percentages of baseline intakes on representative days during the 4-week treatment period (days D1/2-D27/28) and during the 2-week drug-free posttreatment period (days P1/2-P13/14).

significant effect on the food intake of rats in the EB condition group ($F(3,18) = 7.88, p = .001$). By days 7-8 of treatment, mean food intake had returned to baseline levels in all d-fenfluramine-treated rats except those in the DIO condition group ($F(3,18) = 42.64, p < .001$). Food intakes remained suppressed in d-fenfluramine-treated rats in the DIO group on days 13-14, but had returned to baseline levels by days 27-28.

There was a significant interaction between the treatment and day of treatment variables ($F(3,18) = 6.67, p = .003$), but neither the treatment by condition interaction ($F(3,18) = 1.67, p = .208$) nor the condition by day interaction ($F(9,54) = .543, p > .50$) were significant.

Food intakes during the 2-week posttreatment period, expressed as percentages of baseline intakes, were analyzed by 3-way ANOVA with repeated measures design. Days 1-2, 7-8 and 13-14 of the posttreatment period were incorporated into the analysis comparing food intakes of rats previously treated with d-fenfluramine to those of untreated control rats from each of the four dietary or surgical conditions.

Rats in the different dietary or surgical condition groups consumed different percentages of baseline food intakes during the posttreatment period ($F(3,18) = 5.30, p = .008$). This difference was most evident in rats from the EB and DIO condition groups. The mean intake of these rats during the posttreatment period was

approximately 34% above their corresponding baseline intakes; however, there was no significant difference in the percentage intakes of untreated control rats and rats that had received d-fenfluramine ($F(1,6) = 3.65, p = .10$). There was a significant difference between food intakes on the different days ($F(2,12) = 3.77, p = .05$), but this did not reflect a consistent trend in any of the treatment groups. The interaction between the condition and day of treatment variables was not significant ($F(6,36) = 1.8, p = .20$).

In order to determine if d-fenfluramine treatment differentially affected consumption of the dietary components in the cafeteria diet, the percentages of total caloric intake contributed by each component were analyzed. During the 4-week treatment period, untreated control rats in the DIO group consumed a daily average of 11% of their total calories in Chow, 31% in cookie and 58% in sweetened milk (Figure 4). Fenfluramine treatment significantly suppressed the percentage of total calories eaten in Chow (Student's 2-tail t-test, $p = .02$), whereas the percentage of the total intake consumed in milk was increased ($p = .01$).

Body weight. Group mean weekly body weight changes and cumulative weight change during the 4-week treatment and 2-week posttreatment periods are shown for rats in the OVX (Figure 5), EB (Figure 6), DIO (Figure 7) and CHOW dietary or surgical condition groups (Figure 8). During the 4-week drug treatment period,

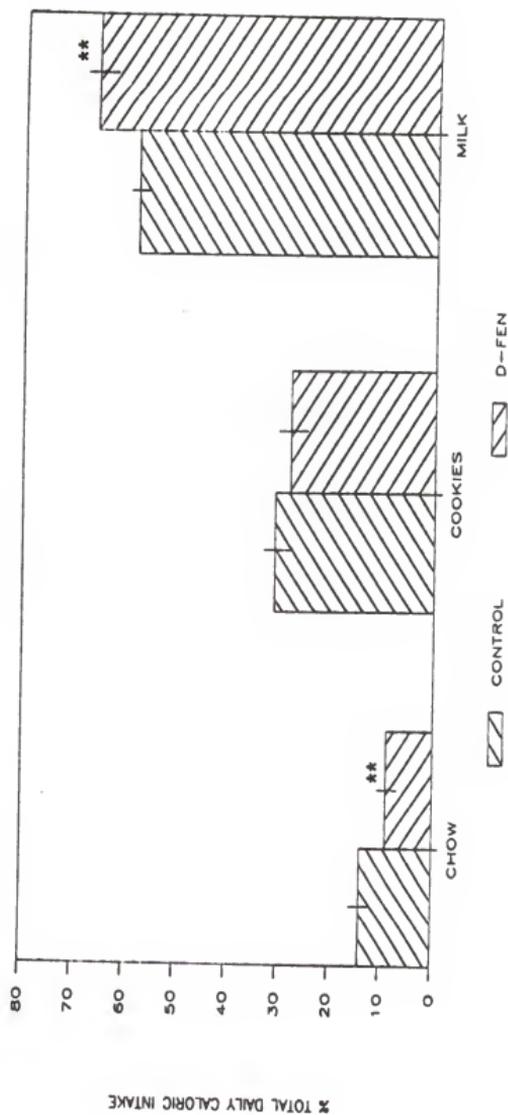


Figure 4. Effects of d-fenfluramine treatment on dietary components of a cafeteria diet. Shown are the percentages of total caloric intake contributed by each food in the cafeteria diet of d-fenfluramine-treated rats (D-FEN) and untreated control rats in the DIO condition group.
 ** Different from control intake of the specific dietary component, Student's 2-tailed t-test, $p \leq .02$.

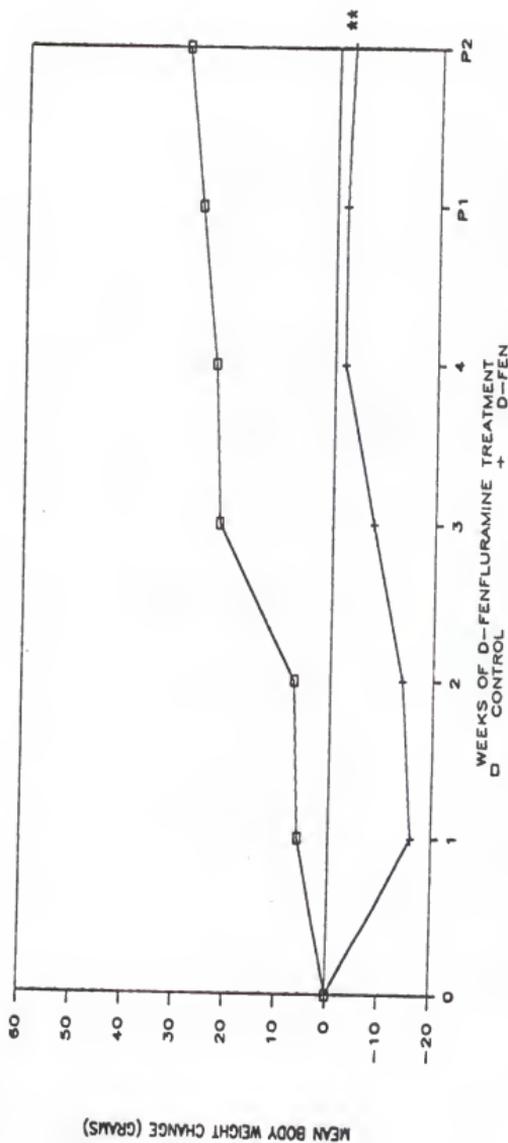


Figure 5. Effects of d-fenfluramine treatment on body weights of ovariectomized (OVX) rats. Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN) and untreated controls in the OVX condition group. Cumulative mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4) and during the 2-week drug-free posttreatment period (weeks P1-P2).

** Total body weight change during the 4-week treatment period different from corresponding group untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

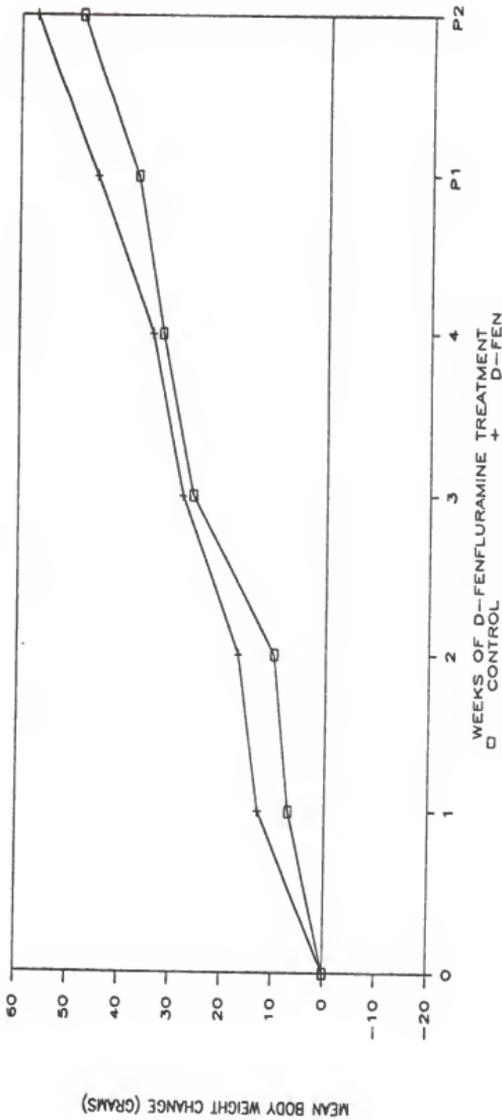


Figure 6. Effects of d-fenfluramine treatment on body weights of ovariectomized rats that received estradiol replacement (EB). Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN) and untreated controls in the EB condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4) and during the 2-week drug-free posttreatment period (weeks P1-P2).

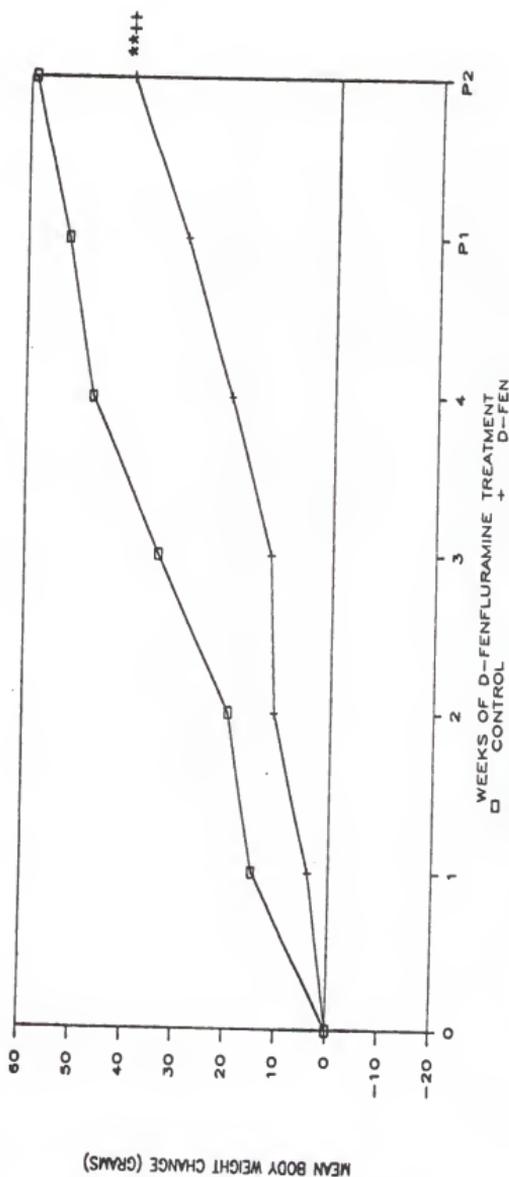


Figure 7. Effects of d-fenfluramine treatment on body weights of dietary obese (DIO) rats. Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN) and untreated controls in the DIO condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4) and during the 2-week drug-free posttreatment period (weeks P1-P2).

** Total weight change during the treatment period (weeks 1-4) different from untreated control group, $p < .01$, Newman-Kuel's post hoc t-test.

†† Total weight change during the drug-free posttreatment period (weeks P1-P2) different from untreated control group, $p < .01$.

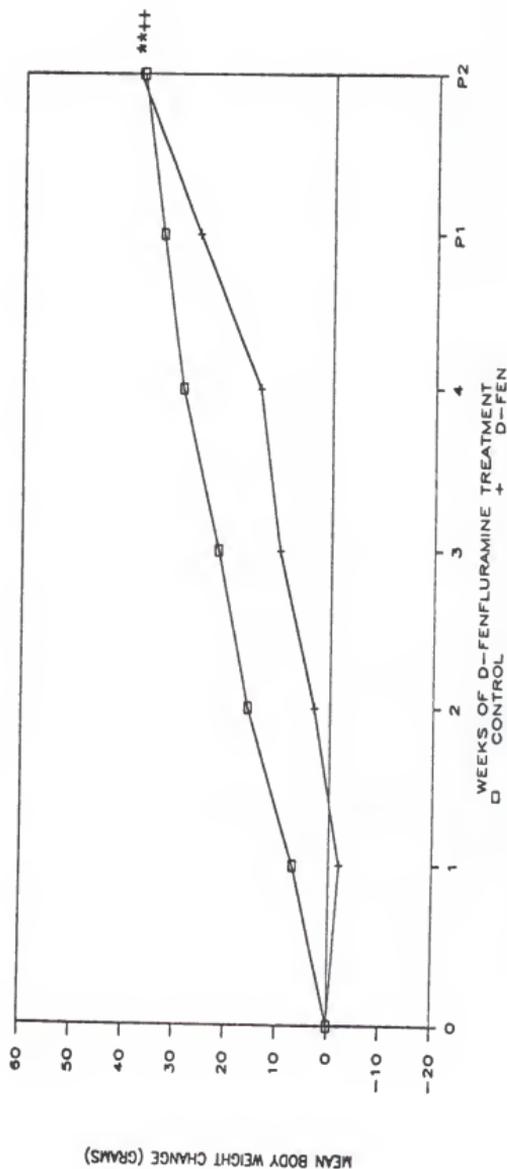


Figure 8. Effects of d-fenfluramine treatment on body weights of unoperated Chow-fed (CHOW) rats. Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN) and untreated controls in the CHOW condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4) and during the 2-week drug-free posttreatment period (weeks P1-P2).

** Total weight change during the treatment period (weeks 1-4) different from untreated control group, $p < .01$, Newman-Keuls's post hoc t-test.

++ Total weight change during the drug-free posttreatment period (weeks P1-P2) different from untreated control group, $p < .01$.

untreated control rats in the DIO group gained more weight than control rats in the CHOW and OVX groups and control rats in the EB group gained more weight than control rats in the OVX group. Rats from the DIO and EB condition groups that received d-fenfluramine also gained more weight than drug-treated rats from the CHOW group, whereas d-fenfluramine-treated rats in the OVX group lost weight during the treatment period (2-way ANOVA, $F(3,75) = 9.74$, $p < .001$). Continuous administration of d-fenfluramine for 4 weeks resulted in a weight loss in OVX rats, significantly reduced weight gain in DIO and CHOW rats and had no significant effect on the body weights of rats in the EB group ($F(1,75) = 11.36$, $p < .001$). The interaction between the dietary or surgical condition variable was not significant ($F(3,75) = 1.78$, $p = .16$).

During the 2-week drug-free posttreatment period, rats in the DIO and CHOW condition groups that had received d-fenfluramine gained significantly more weight than untreated controls in the corresponding groups ($F(1,48) = 4.72$, $p = .03$). Rats in the OVX condition group that had been treated with d-fenfluramine lost a small amount of weight during the posttreatment period; however, their total weight change during the 2-week posttreatment period was not significantly different from that of untreated control rats in the OVX group. There were no significant treatment effects on the mean weight change of rats in the EB condition group during this period.

Food efficiency. Food efficiency was estimated from [total weight gain (grams)/total kilocalories food ingested] during the 4-week treatment period and during the 2-week drug-free posttreatment period for rats from the EB, DIO and CHOW dietary or surgical condition groups and in untreated control rats from the OVX, EB, DIO and CHOW condition groups (Table 4). Fenfluramine-treated rats in the OVX condition group lost weight during both the treatment and posttreatment periods; therefore, it was not possible to estimate food efficiency for the rats in that group from the formula used herein.

Table 4. Effects of d-Fenfluramine on Food Efficiency

Treatment	OVX	EB	DIO	CHOW
Control	6.77 ± 1.05	12.45 ± 3.89+	6.67 ± 1.37	6.36 ± 1.58
D-FEN(4wk)	—	13.77 ± 2.72+	7.73 ± 1.41	7.23 ± 1.49
D-FEN+2wk	—	16.52 ± 4.53+	7.79 ± 2.39	7.44 ± 1.97

Shown are group mean ± S.E.M. food efficiency estimated from [weight gained (grams)/kilocalorie food ingested] during the 4-week d-fenfluramine treatment period (D-FEN) and during the 2-week drug-free posttreatment period for rats from the EB, DIO and CHOW condition groups. Data are shown for untreated control rats in the OVX, EB, DIO and CHOW dietary or surgical condition groups.

+ Different from CHOW untreated control group, Newman Kuel's post hoc t-test, $p < .05$.

The estimated food efficiency was significantly higher in untreated control rats from the EB condition group than in control rats from the OVX, DIO and CHOW dietary or surgical conditions (2-way ANOVA, $F(2,49) = 5.78$, $p < .001$). Administration of d-fenfluramine had no significant effect on the estimated food efficiency of rats in the EB, DIO and CHOW dietary or surgical condition groups ($F(1,49) = 2.45$, $p = .12$).

During the 2-week posttreatment period, the estimated food efficiency of untreated control rats in each of the dietary or surgical condition groups was similar to their food efficiency during the preceding 4-week period. Untreated control rats in the EB condition group had higher [weight gain/kilocalorie ingested] ratios than did untreated control rats in the other condition groups ($F(2,49) = 6.40$, $p < .001$). There was no significant effect of previous d-fenfluramine treatment on food efficiency ($F(1,49) < 1.00$, $p > .25$).

Effects of d-Fenfluramine on Adipocyte Size

One measure of adiposity is adipocyte size which can be estimated from cell diameter or volume. A decrease in lipid storage associated with weight loss may be reflected in a decrease in adipocyte size in one or more fat depots. To determine if weight loss associated with chronic d-fenfluramine treatment is a result of decreased lipid storage, the sizes of white and brown adipocytes were measured in d-fenfluramine-treated rats that were

killed at the end of the drug treatment period or after a 2-week drug-free period and in untreated controls from each of the four dietary or surgical condition groups. The sizes of inguinal white adipocytes (Table 5) and interscapular brown adipocytes (Table 6) were estimated from measurements of cell diameters.

Table 5. Effects of d-Fenfluramine on Inguinal Adipocyte Size

Treatment	OVX	EB	DIO	CHOW
Control	.43 ± .020	.29 ± .002++	.44 ± .010	.43 ± .010
D-FEN(4wk)	.34 ± .010**	.30 ± .001	.24 ± .010**	.23 ± .005**
D-FEN+2wk	.38 ± .001*	.30 ± .010	.34 ± .010**!	.39 ± .010*!

Shown are group mean ± S.E.M. inguinal white adipocyte diameters (micrometers) at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values for rats from each of the dietary or surgical condition groups.

* Different from untreated controls in the corresponding condition group, $p < .05$, Newman Kuel's post hoc t-test.

** Different from corresponding untreated control group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group.

++ Different from CHOW control group, $p < .01$.

The mean diameters of inguinal adipocytes were similar in untreated control rats from the OVX, DIO and CHOW groups, but the mean adipocyte diameter was significantly lower in rats from the EB group (2-way ANOVA, $F(3,23) = 7.00$, $p < .001$). Administration of d-fenfluramine for 4 weeks significantly reduced inguinal

adipocyte diameters in rats from the OVX, DIO and CHOW groups, but had no effect on the diameters of inguinal adipocytes of rats from the EB condition group ($F(1,23) = 38.00, p < .001$). There was a significant interaction between the group condition and treatment variables ($F(3,23) = 13.00, p < .001$).

By 2 weeks after the termination of d-fenfluramine treatment, the group mean inguinal adipocyte diameters of rats in the OVX and EB dietary or surgical condition groups were comparable to the group mean adipocyte diameters of untreated control rats in those condition groups; however, the group mean adipocyte diameters of rats from the DIO and CHOW condition groups that had received d-fenfluramine remained significantly below adipocyte diameters of control rats in their corresponding condition groups ($F(2,34) = 39.00, p < .001$). The differences between the adipocyte diameters of rats from the four dietary or surgical condition groups remained significant ($F(3,34) = 8.00, p < .001$) and the interaction between the group condition and treatment variables also remained significant ($F(3,34) = 14.00, p < .001$).

Brown adipocyte diameters were not significantly different among the dietary or surgical condition groups (2-way ANOVA, $F(3,23) < 1.00, p > .25$) and were not significantly affected by d-fenfluramine treatment ($F(1,23) = < 1.00, p > .25$).

After the 2-week drug-free period, there were no significant differences in brown adipocyte diameters of d-fenfluramine-treated

rats compared to diameters of adipocytes sampled from untreated controls or d-fenfluramine-treated rats killed at the end of the treatment period ($F(3,34) < 1.00, p > .25$).

Table 6. Effects of d-Fenfluramine on Interscapular Brown Adipocyte Size

Treatment	OVX	EB	DIO	CHOW
Control	.08 ± .020	.03 ± .004	.06 ± .015	.04 ± .010
D-FEN(4wk)	.05 ± .010	.03 ± .002	.05 ± .005	.05 ± .020
D-FEN+2wk	.09 ± .020	.03 ± .001	.05 ± .006	.05 ± .005

Shown are group mean ± S.E.M. interscapular brown adipocyte diameters (micrometers) at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values from rats in each of the 4 dietary or surgical condition groups.

Effects of d-Fenfluramine on Brain 5-Hydroxytryptamine (5-HT) and Dopamine (DA)

The most studied aspect of fenfluramine's actions is its effects on brain 5-hydroxytryptamine (5-HT). This action often has been used to explain fenfluramine's effects on food intake. There is evidence that brain 5-HT has a role in feeding behavior; however, the nature of this role is not clearly defined and much of the evidence is controversial. Fenfluramine also affects brain

dopamine (DA) and there is evidence that DA may be involved in feeding behavior; but again, the exact nature of this is unclear.

The effects of d-fenfluramine treatment on concentrations of 5-HT, 5-HIAA, DA and DOPAC in rat telencephalon and hypothalamus were measured by high pressure liquid chromatography with electrochemical detection (HPLC-ED). Regional concentrations of each monoamine and the ratios of metabolite/neurotransmitter were compared between d-fenfluramine-treated rats and untreated controls by Student's 2-tailed t-test.

Brain 5-HT concentrations are shown in Table 7. Continuous delivery of d-fenfluramine by osmotic minipump for 4 weeks at a dosage of 3 mg/kg/day had no significant effects on the concentrations of 5-HT ($p > .10$) or 5-HIAA ($p > .10$) or on the ratio of 5-HIAA/5-HT ($p > .10$) in the telencephalon. There were significant increases in the concentrations of 5-HT ($p < .05$) and 5-HIAA ($p < .05$) in the hypothalamus, but the ratio of 5-HIAA/5-HT was not significantly affected ($p > .05$).

The concentrations of brain monoamines in rats that received d-fenfluramine treatment followed by a 2-week drug-free period were compared to those of rats killed at the end of the drug

Table 7. Effects of d-Fenfluramine on Brain Serotonin (5-HT) and 5-HIAA Concentrations

<u>Telencephalon</u>			
Treatment	5-HT	5-HIAA	5-HIAA/5-HT
Control	7.39 ± 0.38	1.49 ± 0.11	$20 \pm 1\%$
D-FEN(4wk)	6.98 ± 0.41	1.13 ± 0.13	$16 \pm 2\%$
D-FEN+2wk	7.56 ± 0.73	1.24 ± 0.09	$18 \pm 2\%$
<u>Hypothalamus</u>			
Treatment	5-HT	5-HIAA	5-HIAA/5-HT
Control	7.51 ± 0.46	2.51 ± 0.20	$34 \pm 2\%$
D-FEN(4wk)	9.48 ± 0.53 *	3.37 ± 0.32 *	$36 \pm 3\%$
D-FEN+2wk	7.49 ± 0.52	2.90 ± 0.21	$39 \pm 2\%$

Shown are group mean \pm S.E.M. concentrations (nanomoles/gram wet tissue) of 5-HT and 5-HIAA in the telencephalon and hypothalamus at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values. Also shown are the ratios of 5-HIAA/5-HT expressed as percentages \pm S.E.M.

* Different from untreated control values, $p < .05$, Newman-Kuel's post hoc t-test.

treatment period and to untreated controls by 1-way ANOVA. In the telencephalon, there were no significant differences among the treatment groups in the concentrations of 5-HT ($F(2,67) > 1.00$, $p > .25$) or 5-HIAA ($F(61) < 1.00$, $p > .25$) or in the ratio of 5-HIAA/5-HT ($F(2,61) < 1.00$, $p > .25$).

Two weeks after the termination of d-fenfluramine treatment, the concentration of 5-HT in the hypothalamus had returned to control levels and thus, was significantly lower than values obtained from rats killed at the end of the drug treatment period ($F(2,75) = 5.00$, $p = .009$). There were no significant differences in the hypothalamic concentrations of 5-HIAA among the three treatment groups ($F(2,71) = 2.66$, $p = .08$) or in the ratio of 5-HIAA/5-HT ($F(2,71) = 1.18$, $p = > .25$).

Brain DA concentrations are shown in Table 8. The concentrations of DA and DOPAC in the telencephalon and hypothalamus and the ratios of DOPAC/DA in those regions were not affected significantly by 4 weeks of d-fenfluramine treatment (Student's 2-tailed t-test, $p > .05$). The 1-way ANOVAs comparing data collected at the end of the 4-week treatment period or at 2 weeks after the termination of treatment and untreated controls were not statistically significant in any case.

Table 8. Effects of d-Fenfluramine on Brain Dopamine (DA) and DOPAC Concentrations

<u>Telencephalon</u>			
Treatment	DA	DOPAC	DOPAC/DA
Control	4.22 ± 0.51	1.10 ± 0.20	$27 \pm 4\%$
D-FEN(4wk)	3.99 ± 0.44	0.87 ± 0.13	$25 \pm 5\%$
D-FEN+2wk	3.94 ± 0.54	1.02 ± 0.31	$23 \pm 5\%$

<u>Hypothalamus</u>			
Treatment	DA	DOPAC	DOPAC/DA
Control	1.51 ± 0.17	0.77 ± 0.08	$60 \pm 4\%$
D-FEN(4wk)	1.42 ± 0.10	1.12 ± 0.29	$68 \pm 6\%$
D-FEN+2wk	1.26 ± 0.20	0.94 ± 0.09	$52 \pm 5\%$

Shown are group mean concentrations (nanomoles/gram wet tissue) of DA and DOPAC in the telencephalon and hypothalamus at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after a 2-week drug-free period (D-FEN + 2 wk) and untreated control values. Also shown are the ratios of DOPAC/DA expressed as percentages \pm S.E.M.

Peripheral Actions of d-Fenfluramine

Fenfluramine may be more effective in producing sustained weight loss in animals with a higher percentage body fat. Comparing data from a number of different studies, we have found a correlation between weight loss and initial body weight with chronic daily drug injections (Rowland and Carlton, 1986). In preliminary studies, we found prolonged anorexia and weight loss in rats made obese by feeding a palatable and varied diet (Carlton and Rowland, 1986). It has been reported that as much 90% of the weight gain observed on similar diets is due to increased lipid storage (Rothwell and Stock, 1979b). We also had preliminary indications that fenfluramine may be more effective in female rats than in males. In rats, as in humans, females generally have higher ratios of adipose to muscle tissue (AMR) than males of comparable body weight. These results suggested that a higher percentage of body fat, rather than increased body weight per se, may be the important factor for explaining the greater efficacy of fenfluramine in heavier animals.

Peripheral actions of fenfluramine may be relevant to its antiobesity actions and also may help explain possible differences in drug efficacy in lean and obese animals. Previous reports on the peripheral actions of fenfluramine have used d,l-fenfluramine primarily, although some of the more recent studies have used d-fenfluramine. Many of the previous studies have been conducted

using high doses of fenfluramine or high in vitro concentrations to examine peripheral drug actions. No previous studies have used a treatment regimen similar to the one used in the current experiments to study peripheral actions of d-fenfluramine.

NPPase activity. The activity of K^+ -nitrophenylphosphatase (NPPase) is an index of the activity of the enzyme (Na^+-K^+)ATPase and hence reflects cellular activity (Swann, 1984a, 1984b). The activity of NPPase (nanomoles/minute/milligram protein) was measured in interscapular brown adipose tissue (BAT) (Table 9) and in gastrocnemius muscle (Table 10) of d-fenfluramine-treated and untreated control rats from each of the four dietary or surgical conditions.

The activity of NPPase in BAT was lower in untreated control rats from the OVX group compared to control rats in the other condition groups (2-way ANOVA, $F(3,34) = 14.83$, $p < .001$). Chronic (4-week) administration of 3 mg d-fenfluramine/kg/day via osmotic minipump significantly increased NPPase activity in BAT of rats from the OVX and CHOW groups, but did not affect the activity of this enzyme significantly in rats from the EB and DIO condition groups ($F(1,34) = 7.59$, $p < .001$). There was no significant interaction between the dietary or surgical condition variable and the treatment variable ($F(3,34) = 2.29$, $p > .25$).

Table 9. Effects of d-Fenfluramine on NPPase Activity in Brown Adipose Tissue

Treatment	OVX	EB	DIO	CHOW
Control	1.44±0.13++	4.28±1.16	5.87±0.64	6.00±1.10
D-FEN(4wk)	5.82±0.43**	5.20±0.88	6.42±0.56	10.73±1.42*
D-FEN+2wk	2.52±0.40*!	5.25±1.15	5.03±0.83	4.19±0.82!

Shown are group mean \pm S.E.M. NPPase activity in brown adipose tissue expressed as nanomoles/minute/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values for rats from the four dietary or surgical condition groups.

* Different from untreated control rats in the corresponding condition group, $p < .05$, Newman Kuel's post hoc t-test.

** Different from corresponding untreated control group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

++ Different from CHOW untreated control group, $p < .01$.

By the end of the 2-week drug-free period, NPPase activity in BAT of rats in the OVX and CHOW condition groups that had been treated previously with d-fenfluramine was significantly lower than NPPase activity at the end of the treatment period ($F(2,56) = 10.41$, $p < .001$). In the OVX condition, the increased NPPase activity associated with d-fenfluramine administration was not reversed completely within 2 weeks, but was significantly lower than NPPase activity measured at the termination of drug treatment. The interaction between the treatment and dietary or surgical condition group variables was significant ($F(6,56) = 2.63$, $p = .02$).

The activity of NPPase in gastrocnemius muscle was significantly decreased in untreated control rats from the OVX and DIO condition groups and significantly increased in control rats from the EB group compared to enzyme activity in control rats from the CHOW group (2-way ANOVA, $F(3,38) = 24.31$, $p < .001$). Four-week administration of d-fenfluramine significantly increased the activity of muscle NPPase in rats from the OVX group ($F(1,38) = 4.59$, $p = .04$), but the increase was not sufficient to raise enzyme activity in these rats to the same level as in untreated control rats from the CHOW group. Administration of d-fenfluramine had no significant effect on muscle NPPase activity in rats from the other dietary or surgical condition groups compared to untreated controls in those groups, thus enzyme activity in d-fenfluramine-treated rats from the DIO group remained below the level of activity in untreated control rats from the CHOW group. There was no significant interaction between the dietary or surgical condition variable and the treatment variable ($F(3,38) < 1.00$, $p > .25$).

At the end of the 2-week drug-free period, NPPase activity in gastrocnemius muscle of rats in the OVX group that had been treated with d-fenfluramine was significantly lower than at the end of drug treatment and was not significantly different from enzyme activity in untreated control rats ($F(2,58) = 9.93$, $p < .001$).

Table 10. Effects of d-Fenfluramine on NNpase Activity in Gastrocnemius Muscle

Treatment	OVX	EB	DIO	CHOW
Control	1.06±0.12++	7.76±1.08++	2.62±0.51++	4.75±0.62
D-FEN(4wk)	2.00±0.21***	5.54±1.65	2.24±0.21++	5.62±0.62
D-FEN+2wk	1.27±0.10!++	5.37±0.31	2.38±0.17++	5.12±1.00

Shown are group mean ± S.E.M. NNpase activity in gastrocnemius muscle expressed as nanomoles/minute/milligram protein at the end of 4 weeks of d-fenfluramine (D-FEN) or after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control rats in the four dietary or surgical condition groups.

** Different from untreated control rats in the corresponding condition group, $p < .01$, Newman-Kuel's post hoc t-test.

++ Different from CHOW untreated control group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

Lipoprotein lipase (LPL) activity. There are two triglyceride lipases in adipose tissue that are the primary regulators of storage and mobilization of lipids. Hormone-sensitive lipase (HSL), the rate limiting enzyme in the breakdown of adipocyte triglycerides to release free fatty acids (FFA) and glycerol, is critically responsive to plasma catecholamines. Lipoprotein lipase (LPL) catalyzes the breakdown of triglycerides from plasma sources providing FFA for reesterification and storage within the adipocyte and is the rate limiting enzyme in the formation of triglycerides in adipocytes (Desai and Hollenberg, 1975; Garfinkel

et al., 1967). This enzyme is also found in other tissues such as cardiac and skeletal muscle, where it catalyzes the hydrolysis of triglycerides to provide FFA for fuel.

The activity of LPL was measured in retroperitoneal and inguinal white adipose tissue and in interscapular BAT (IBAT) in d-fenfluramine-treated rats that were killed at the end of the 4-week treatment period or after a 2-week drug-free period and in untreated control rats from the four dietary or surgical condition groups. Lipoprotein lipase activity is expressed as micromoles FFA/hour/milligram protein in retroperitoneal (Table 11), inguinal (Table 12) and in interscapular brown (Table 13) adipose tissue. The data also were converted to micromoles FFA/hour/milligram tissue; however, the statistical analyses of the two sets of data yielded similar results; therefore, only the former set of data is shown.

The activity of retroperitoneal LPL was significantly lower in untreated control rats from the DIO group and higher in control rats from the OVX condition group compared to control rats in the CHOW group (2-way ANOVA, $F(3,35) = 3.11$, $p = .05$).

After 4 weeks of d-fenfluramine administration, LPL activity was decreased significantly in all four dietary and surgical condition groups ($F(1,35) = 4.74$, $p = .03$). The decreases in retroperitoneal LPL activity were substantial, ranging from a 38% decrease in the DIO group to a 78% decrease in the OVX group.

There was not a significant interaction between the dietary or surgical condition variable and the treatment variable ($F(3,35) = 1.59, p = .21$).

Table 11. Effects of d-Fenfluramine on LPL Activity in Retroperitoneal White Adipose Tissue

Treatment	OVX	EB	DIO	CHOW
Control	2.27±.62++	0.56±.17	0.36±.03++	0.87±.21
D-FEN(4wk)	0.50±.08**	0.24±.04**	0.26±.04**	0.29±.09**
D-FEN+2wk	1.07±.22**!	0.59±.14!	0.54±.21!	0.49±.14*

Shown are group mean \pm S.E.M. lipoprotein lipase (LPL) activity expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values for rats from the four dietary or surgical condition groups.

* Different from untreated control rats in the corresponding dietary or surgical condition group, $p < .05$, Newman-Kuel's post hoc t-test.

** Different from corresponding untreated control group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group, $P < .01$.

++ Different from CHOW untreated control group, $p < .01$.

At the end of the 2-week drug-free period, LPL activity in retroperitoneal WAT of rats in the DIO and EB groups that had been treated with d-fenfluramine was comparable to enzyme activity in the corresponding control groups; however, LPL activity of rats in the OVX and CHOW groups remained significantly below untreated control levels ($F(2,54) = 3.41, p = .03$).

Lipoprotein lipase activity in inguinal WAT was significantly elevated in untreated control rats from the OVX group compared to control rats in the other dietary or surgical condition groups ($F(3,46) = 9.87, p < .001$). Administration of d-fenfluramine for 4 weeks had no significant effect on inguinal LPL activity in any of the dietary or surgical condition groups ($F(1,46) < 1.00, p > .25$).

Table 12. Effects of d-Fenfluramine on LPL Activity in Inguinal White Adipose Tissue

Treatment	OVX	EB	DIO	CHOW
Control	1.21 ± .19 ⁺⁺	0.43 ± .13	0.32 ± .05	0.39 ± .05
D-FEN(4wk)	1.20 ± .23	0.51 ± .08	0.38 ± .01	0.38 ± .12
D-FEN+2 wk	0.81 ± .25	0.67 ± .15	0.48 ± .01	0.38 ± .06

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values for rats from the four dietary or surgical condition groups.

⁺⁺ Different from CHOW untreated control group, $p < .01$, Newman Kuel's post hoc t-test.

The activity of LPL in IBAT was significantly higher in untreated control rats from the OVX and DIO groups and significantly lower in control rats from the EB group compared to control rats in the CHOW group (2-way ANOVA, $F(3,33) = 11.00, p < .001$). Administration of d-fenfluramine for 4 weeks had no significant effect on the activity of LPL in BAT ($F(1,33) < 1.00, p > .25$).

There were no significant differences in the activity of LPL in BAT samples taken from d-fenfluramine-treated rats killed after a 2-week drug-free period compared BAT samples taken at the termination of drug treatment or untreated controls ($F(2,48) < 1.00, p > .25$).

Table 13. Effects of d-Fenfluramine on LPL Activity in Interscapular Brown Adipose Tissue

Treatment	OVX	EB	DIO	CHOW
Control	.28±.03++	.07±.01+	.17±.04+	.10±.01
D-FEN(4wk)	.22±.03	.09±.07	.09±.01	.12±.02
D-FEN+2wk	.26±.02	.09±.03	.18±.05	.09±.02

Shown are group mean \pm S.E.M. lipoprotein lipase (LPL) activity expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment or after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) and untreated control values from the four dietary or surgical condition groups.

+ Different from CHOW untreated control group, $p < .05$, Newman-Kuel's post hoc t-test.

++ Different from CHOW untreated control group, $p < .01$.

Carbohydrate and lipid metabolism. The regulation of metabolism is primarily under the control of the autonomic nervous system which regulates the reciprocal processes of energy storage and utilization. Activity of the parasympathetic nervous system stimulates release of insulin and inhibits glucagon release from the endocrine pancreas whereas activation of the sympathetic

nervous system has opposite effects. These hormones have direct and, in general, opposing actions at the level of cellular metabolism.

Insulin is released in response to high plasma levels of nutrients such as after a meal. It stimulates glucose utilization and storage as glycogen in liver and muscle. Insulin also stimulates uptake and storage of FFA. Hyperinsulinemia is commonly found in a majority of obese animal models and in humans with longstanding obesity. The result of persistent hyperinsulinemia associated with obesity may be the development of insulin receptor insensitivity and Type II diabetes mellitus which constitutes a major health risk for the chronically obese.

The pituitary-adrenal system also has important actions in the regulation of metabolism particularly in times of physiological or psychological stress. The adrenocorticoids have actions similar to the sympathetic neurotransmitters as well as direct effects at the cellular level. Hypercorticism is a characteristic of several animal models of obesity and is found in humans with longstanding obesity.

Plasma insulin concentrations. Plasma insulin concentrations, expressed as microunits insulin/milliliter plasma, of rats treated with d-fenfluramine for 4 weeks and untreated control rats from the four dietary or surgical condition groups are shown in Figure 9. Also shown in Figure 9 are plasma insulin

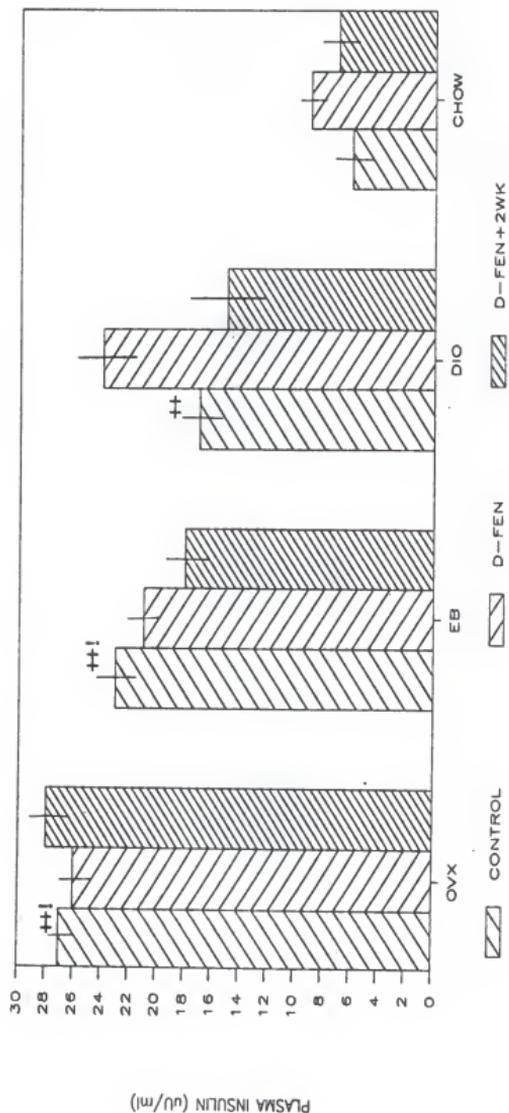


Figure 9. Effects of d-fenfluramine treatment on plasma insulin concentrations. Plasma insulin concentrations, expressed in microunits insulin/milliliter plasma ($\mu\text{U}/\text{ml}$), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK) and untreated control values for rats from the four dietary or surgical condition groups.

! Different from DIO group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

!+ Different from CHOW group, untreated control, $p < .01$.

concentrations of rats treated with d-fenfluramine followed by a 2-week drug-free period. Plasma samples were obtained from trunk blood collected between 08:00 and 14:00 hours immediately after decapitation of rats that were fasted overnight (12-18 hours).

Plasma insulin concentrations in untreated control rats from the OVX, EB and DIO groups were elevated significantly compared to insulin concentrations in control rats from the CHOW group and insulin concentrations of rats from the DIO group were slightly, but significantly, less than insulin concentrations of rats from the OVX and EB groups (2-way ANOVA, $F(3,39) = 11.08$, $p < .001$). Administration of d-fenfluramine for 4 weeks had no significant effect on plasma insulin concentrations of rats in the four dietary or surgical conditions ($F(1,39) < 1.00$, $p > .25$).

There were no significant differences in plasma insulin concentrations of d-fenfluramine-treated rats killed after a 2-week drug-free period compared to insulin concentrations of rats killed at the end of the treatment period or untreated control rats ($F(2,59) < 1.00$, $p > .25$).

Plasma glucose concentrations. Plasma glucose concentrations, expressed as milligrams glucose/deciliter plasma, of d-fenfluramine-treated rats killed at the end of the 4-week treatment period or after a 2-week drug-free period and untreated control rats from the four dietary or surgical condition groups are shown in Figure 10. Aliquots for glucose analyses were taken from the

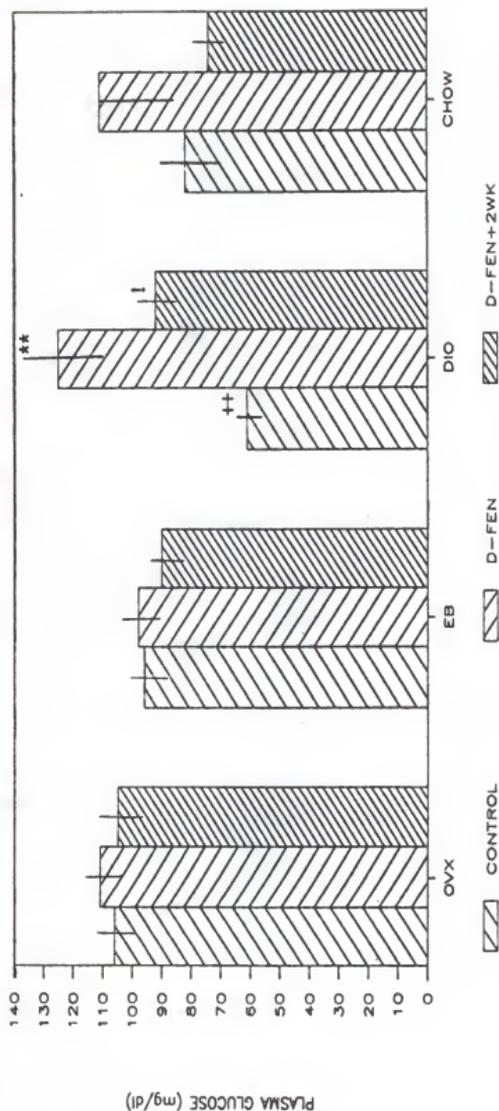


Figure 10. Effects of d-fenfluramine treatment on plasma glucose concentrations. Plasma glucose concentrations, expressed in milligrams glucose/deciliter plasma (mg/dl), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK) and untreated control values for rats from the four dietary or surgical condition groups.

** Different from untreated control values for the corresponding dietary or surgical condition group, $p < .01$, Newman-Kuel's post hoc t-test.

! Different from corresponding D-FEN group, $p < .01$.

++ Different from CHOW group, untreated control, $p < .01$.

same plasma samples that were used for measurement of plasma insulin concentrations.

Plasma glucose concentrations were significantly lower in untreated control rats from the DIO group compared to control rats from the other dietary or surgical condition groups (2-way ANOVA, $F(3,43) = 5.77, p = .002$). Rats from the DIO group that received d-fenfluramine for 4 weeks had significantly higher plasma glucose concentrations compared to untreated control rats from the DIO group ($F(1,43) = 12.02, p = .001$). Plasma glucose concentrations of rats from the other dietary or surgical conditions generally were increased by d-fenfluramine treatment although the increases were not statistically significant. The interaction between the condition and treatment variables was significant ($F(3,43) = 10.45, p < .001$).

Plasma glucose concentrations of d-fenfluramine-treated rats in the DIO group that were killed after a 2-week drug-free period were significantly lower than glucose concentrations of rats killed at the termination of d-fenfluramine treatment ($F(2,62) = 9.90, p < .001$). The interaction between the dietary or surgical condition and treatment variables was significant in this comparison also ($F(6,62) = 3.16, p = .009$).

Plasma triglyceride concentrations. Plasma triglyceride concentrations (Figure 11), expressed as milligrams triglyceride/deciliter plasma, are shown for d-fenfluramine-treated

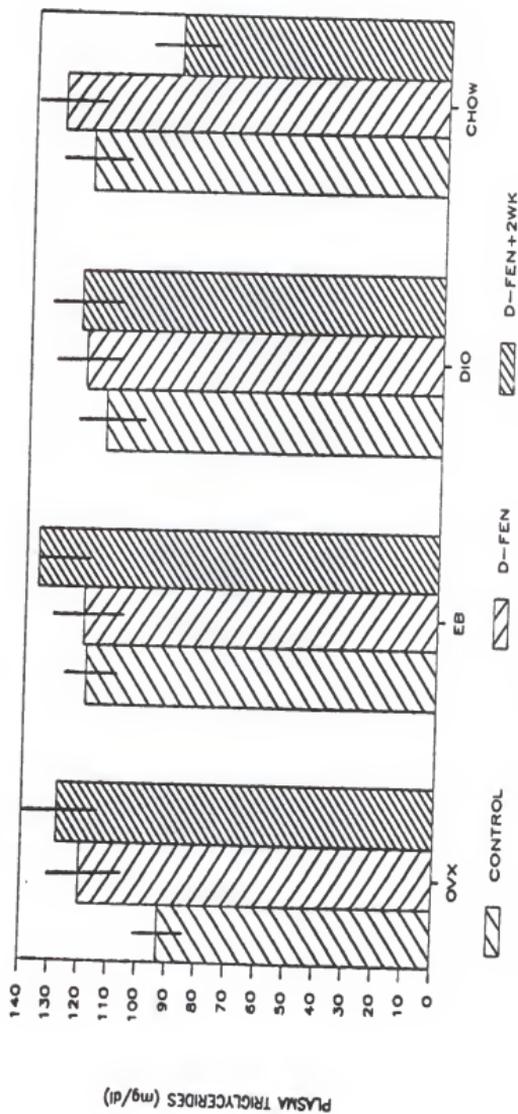


Figure 11. Effects of d-fenfluramine treatment on plasma triglyceride concentrations. Plasma triglyceride concentrations, expressed in milligrams triglycerides/milliliter plasma (mg/dl), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK) and untreated control values for rats from the four dietary or surgical condition groups.

rats killed at the end of the 4-week treatment period or after a 2-week drug-free period and untreated control rats from the four dietary or surgical condition groups. Triglyceride concentrations were measured in aliquots of the same plasma samples that were used for the preceding assays.

Plasma triglyceride concentrations were not significantly different among untreated control rats from the four dietary or surgical condition groups (2-way ANOVA, $F(3,34) = 1.25$, $p > .25$). There was no significant effect of the 4-week d-fenfluramine treatment on plasma triglyceride concentrations ($F(1,34) = 2.70$, $p = .10$).

Plasma triglyceride concentrations of d-fenfluramine-treated rats killed after a 2-week drug-free period were not significantly different from triglyceride concentrations of rats killed at the end of the drug treatment period or of untreated control rats in any of the four dietary or surgical condition groups ($F(2,51) = 2.13$, $p = .12$).

Plasma corticosterone concentrations. Plasma corticosterone concentrations, expressed as micrograms corticosterone/deciliter plasma, of d-fenfluramine-treated rats killed at the end of the 4-week treatment period or after a 2-week drug-free period and in untreated control rats from the four dietary or surgical condition groups are shown in Figure 12. Corticosterone concentrations were

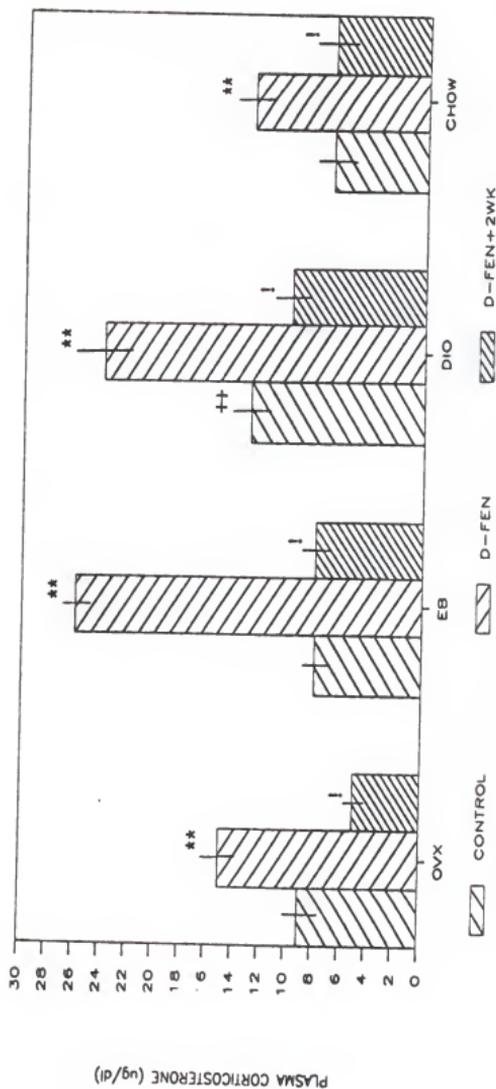


Figure 12. Effects of d-fenfluramine treatment on plasma corticosterone concentrations. Plasma corticosterone concentrations, expressed in micrograms corticosterone/milliliter plasma (ug/ml), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK) and untreated control values for rats from the four dietary or surgical condition groups.

** Different from corresponding group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.
 †† Different from CHOW group, untreated control, $p < .01$.

measured in aliquots of the same plasma samples that were used for the preceding assays.

Plasma corticosterone concentrations were elevated significantly in untreated control rats from the DIO group compared to control rats in the CHOW group (2-way ANOVA, $F(3,42) = 4.09$, $p = .01$). Administration of d-fenfluramine for 4 weeks produced significant elevations in plasma corticosterone concentrations in rats in all four dietary or surgical condition groups compared to the corresponding group controls ($F(1,42) = 27.02$, $p < .001$). There was no significant interaction between the condition and treatment variables ($F(3,42) = 1.8$, $p = .16$).

By 2 weeks after the termination of d-fenfluramine treatment, plasma corticosterone concentrations had returned to control levels; therefore, corticosterone concentrations of d-fenfluramine-treated rats that were killed 2 weeks after the termination of drug treatment were significantly lower than corticosterone concentrations of rats killed at the end of the drug treatment period ($F(2,64) = 21.37$, $p < .001$). The interaction between the treatment and dietary or surgical group variables was not statistically significant ($F(6,64) = 1.87$, $p = .07$).

Experiment 1: Discussion

Two animal models of obesity were used in the present experiments: diet-induced obesity produced by feeding a varied, palatable diet (DIO) and obesity developed subsequent to ovariectomy

(OVX). The model of diet-induced obesity seems to be relevant to many of cases of human obesity. Palatability and food composition are extremely important determinants of food intake and availability of a variety of highly palatable foods apparently can override early satiety signals. This model also may be relevant in some regards to special cases of overeating such as Bulimia Nervosa, which is characterized by ingestion of large amounts of palatable (high carbohydrate, high fat) foods.

Ovariectomy rapidly and reliably produces weight gain and obesity in rats that can be reversed by administration of physiological levels of estrogen. Females of many species, including humans and rats, have a higher percentage of body fat, the degree and disposition of which is affected by ovarian hormones (Wade and Gray, 1979). Females also have a greater ability to conserve energy stores and alter food efficiency. Naturally occurring or experimentally induced fluctuations in the levels of circulating gonadal hormones produce changes in body weight and fat content, as well as changes in relevant behaviors such as food intake and voluntary exercise (Wade and Gray, 1979).

Features common to these and other animal obesity models and possible causes of obesity include (a) energy imbalance due to increased food intake, decreased metabolic rate, decreased thermogenesis or increased efficiency of metabolism; (b) increased adiposity due to hyperplasia and/or hypertrophy of adipose tissue;

(c) hyperinsulinemia and insulin resistance; (d) enhanced lipid accumulation due to increased lipogenesis and/or decreased lipolysis and (e) hyperlipidemia.

Feeding of a varied, palatable diet sustained increases in food intakes and body weights of female Sprague-Dawley rats. The mean baseline daily caloric intake of rats that were fed a modified cafeteria diet consisting of Chow, cookies and sweetened milk was 53% higher than that of rats maintained on Purina Chow pellets alone. Thus diet-induced obesity was maintained by high caloric intake; however, this was not the case for ovariectomy-induced obesity. The baseline caloric intake of Chow-fed ovariectomized rats was not significantly different from that of unoperated Chow-fed rats.

After 10 weeks on the modified cafeteria diet, rats had 25% higher body weights than unoperated Chow-fed rats. During the 10-week pretreatment period, ovariectomized, Chow-fed rats gained almost exactly the same amount of weight as dietary obese rats. Food intakes were not measured during the pretreatment interval except for the baseline measures taken in the final week before d-fenfluramine treatment began. It is possible that ovariectomized rats had higher caloric intakes during part of the pretreatment period; however, a high caloric intake was not necessary to maintain the ovariectomy-induced obesity.

The effects of ovariectomy on body weight and food intake have been characterized in two stages: an initial, dynamic phase of weight gain during which food intake is increased and body weight increases rapidly, and a second "plateau" phase during which food intake returns to preoperative levels and body weight stabilizes at a higher level (Wade and Gray, 1979).

Administration of a low dose of estradiol benzoate (EB) (2 ug/day) to ovariectomized rats decreased the mean baseline daily caloric intake by 17% compared to ovariectomized rats that did not receive estradiol replacement. This relatively slight decrease in food intake does not seem to account for the larger difference in body weight between the two groups. During the 10-week pretreatment period that followed the ovariectomy surgeries, the weight gain of ovariectomized rats that received estradiol replacement was only 35% that of ovariectomized rats that did not receive estradiol.

Administration of estrogen initially produces hypophagia and weight loss, but after a few weeks, hypophagia subsides and the rate of weight gain returns to normal (Wade and Gray, 1979). The hyperphagia and dynamic weight gain in ovariectomized rats follows a similar pattern. The implications for set-point theory are clear: estradiol reduces food intake and body weight until a lower set-point level is attained or ovariectomy elevates food intake and body weight until a higher set-point level is reached.

Both exogenously administered estrogen and naturally occurring peaks in circulating estrogen produce hypophagia and weight loss. By far the most pronounced effects of estrogen on body composition are on fat content. Following ovariectomy, carcass fat content may double and this can be reversed by administration of estradiol benzoate (Roy and Wade, 1977). The effects of estrogen on body weight cannot be accounted for by its effects on food intake or hyperactivity (Roy and Wade, 1977). Estrogen has effects on a number of metabolic processes that could be related to its effects on food intake and body weight. These have been reviewed by Wade and Gray, (1979). (1) Effects on carbohydrate metabolism include elevation of circulating insulin, increased insulin synthesis and receptor sensitivity. (2) Effects on lipid metabolism including elevation of plasma triglycerides, are probably related to suppression of LPL activity and may involve receptor protein synthesis. (3) Effects on the hypothalamus may be direct: cytoplasmic [³H]estradiol binding sites have been located in the hypothalamus, and diencephalic estrogen implants decrease food intake and increase activity. (4) Actions of estrogen at the anterior pituitary include stimulated release of hormones involved in metabolism. (5) Effects on adipose tissue may be direct: estrogen binding sites are present in abdominal, subcutaneous and brown adipose tissue.

Effects of d-Fenfluramine on Food Intake and Body Weight

The effects of 4 weeks of continuous administration of 3 mg d-fenfluramine/kg/day via osmotic minipumps were assessed in female Sprague-Dawley rats from the four dietary or surgical condition groups: obese ovariectomized (OVX); ovariectomized with estradiol benzoate replacement (EB); dietary obese (DIO) and unoperated Chow-fed (CHOW). The effects of this treatment regimen on food intake, body weight and food efficiency were compared to untreated control rats in each the four dietary or surgical condition groups during the 4-week treatment period.

Food intakes of d-fenfluramine-treated rats and untreated controls from each of the four dietary or surgical conditions were measured at 2-day intervals throughout the 4-week drug treatment period. On the first 2 days of administration, d-fenfluramine treatment suppressed food intake of ovariectomized and dietary obese rats and unoperated Chow-fed rats by a similar degree (52-63%); however, d-fenfluramine had no significant effect on the food intake of estradiol-treated rats, even on the first 2 days of administration.

By days 7-8 of the treatment period, mean food intakes had returned to baseline levels in all d-fenfluramine-treated rats except the dietary obese rats. The mean daily caloric intake of d-fenfluramine-treated rats in the DIO condition group remained at approximately 75% of baseline intake on days 7-8 and 13-14 of

chronic drug treatment, but had returned to baseline levels by days 27-28. There was a significant interaction between the treatment and day of treatment variables since food intake had returned to baseline levels in all except the DIO group within 7 days with chronic d-fenfluramine administration.

The greater efficacy of d-fenfluramine in reducing food intake of dietary obese rats replicates preliminary studies in which 4-week administration of 3 mg d-fenfluramine/kg/day via osmotic minipump reduced food intake of rats fed a similar diet by 25% through the third week of treatment, whereas food intake of Chow-fed rats returned to baseline levels within a few days (Carlton and Rowland, 1986).

The efficacy of d-fenfluramine in reducing food intake in the different dietary or surgical condition groups was more closely related to baseline food intake than body weight: the effects of d-fenfluramine on food intake of ovariectomized rats was not affected to a greater degree than that of unoperated Chow-fed rats, although their body weights at the beginning of the d-fenfluramine treatment period were comparable to body weights of dietary obese rats. The baseline food intake of dietary obese rats was 53% higher than Chow-fed rats; therefore, the 25% reduction in food intake of these rats that was associated with d-fenfluramine administration was not sufficient to decrease their mean daily caloric intake to the level of Chow-fed rats.

During the 2-week posttreatment period, food intakes of rats that had received d-fenfluramine were compared to food intakes of untreated controls from each of the four dietary or surgical conditions. Rats in the different dietary or surgical condition groups consumed different percentages of baseline food intakes during this period. This difference was most evident in rats from the EB and DIO condition groups in which both control and previously d-fenfluramine-treated rats had food intakes that were approximately 34% above their corresponding baseline intakes. There were no significant effects of previous d-fenfluramine treatment on posttreatment food intake.

In order to determine if d-fenfluramine treatment differentially affected consumption of the various dietary components in the cafeteria diet, the percentage of total caloric intake contributed by each component was measured. During the 4-week treatment period, untreated control rats in the DIO condition group consumed a daily average of 11% of their total calories in Chow, 31% in cookie and 58% in sweetened milk. Fenfluramine treatment significantly suppressed the percentage of total calories eaten in Chow whereas the percentage of the total intake consumed in milk was increased.

The effect of d-fenfluramine on the food intake of dietary obese rats was to decrease total caloric intake by reducing intake of all of the dietary components, but by different degrees. The

percentage of total calories consumed as Chow was decreased whereas the percentage consumed in milk was increased. A possible explanation is that d-fenfluramine, at least when acutely administered, slows gastric emptying of solids (e.g. Chow) but not liquids (Rowland and Carlton, 1985). This may be related to long-term effects of d-fenfluramine on food intake. Taste aversion develops to specifically flavored solid foods associated with d-fenfluramine administrations and, to a much lesser degree, to flavored liquids paired with d-fenfluramine (Carlton and Rowland, unpublished observations). It may be that gastric distension caused by delayed emptying of food from the stomach results in discomfort and hence, development of a learned aversion to foods, particularly solid foods, associated with administration of d-fenfluramine. This explanation does not account for the fact that the percentage intake of cookies was not decreased by d-fenfluramine since they are a solid food. The most likely explanation involves palatability of the different foods. When food intake is decreased by d-fenfluramine, the bland Chow is affected most strongly while the sweeter, more palatable foods are spared. This does not rule out an explanation involving taste aversion to solid foods paired with d-fenfluramine; however, it does suggest that palatability may override mechanisms that would ordinarily decrease intake of a specific food.

The effects of d-fenfluramine on the different dietary components argues against the hypothesis that fenfluramine works via a brain 5-HT mechanism to selectively suppress carbohydrate intake while sparing protein intake (e.g. Wurtman and Wurtman, 1984). The evidence for this popular hypothesis is not convincing and has been challenged (e.g. Blundell and Hill, 1987; Fernstrom, 1987; Rowland and Carlton, 1986). In the present experiment, the sweetened lowfat milk was 20% protein whereas the Chow and cookies were 23% and 5% protein respectively. The carbohydrate content of the sweetened milk was 80%; the cookie, 70% and the Chow, 50%. Clearly in this experiment, carbohydrate intake was not suppressed to a greater extent than protein intake.

During the 4-week drug treatment period, untreated dietary obese rats gained more weight than untreated obese ovariectomized or unoperated Chow-fed rats and estradiol-treated rats gained more weight than obese ovariectomized rats. Dietary obese and estradiol-treated rats that received d-fenfluramine also gained more weight than drug-treated, unoperated Chow-fed rats whereas d-fenfluramine-treated ovariectomized rats lost weight during the treatment period. Continuous administration of d-fenfluramine for 4 weeks resulted in a weight loss in ovariectomized rats, significantly reduced weight gain in dietary obese and unoperated, Chow-fed rats and had no significant effect on the body weights of estradiol-treated rats.

Fenfluramine was clearly more effective in reducing the body weights of ovariectomized rats than in dietary obese and unoperated Chow-fed rats whereas it was totally ineffective in reducing body weights of estradiol-treated rats. It is unclear why d-fenfluramine was less effective in reducing body weights of dietary obese rats than in ovariectomized rats since the average body weight of the two groups was comparable upon initiation of treatment. The d-fenfluramine-induced reduction in food intake was prolonged in dietary obese rats compared to other groups including ovariectomized rats; however, as noted above, the effect was not sufficient to reduce daily caloric intake dietary obese rats to a level comparable to that Chow-fed rats in the OVX, EB and CHOW dietary or surgical condition groups. This implies that the availability of a palatable diet may override mechanisms that reduce food intake and body weight. It is possible that the ovariectomized rats are particularly sensitive to some metabolic actions of d-fenfluramine. If indeed fenfluramine is more effective in females the reasons may be related; thus, the drug's effectiveness may be influenced by hormonal status.

The lack of effect of d-fenfluramine administration on food intake and body weight of ovariectomized rats receiving estradiol replacement and the increased efficacy in ovariectomized rats not receiving estradiol are consistent with a set-point model: the drug is more effective in reducing body weight of ovariectomized

rats maintaining a higher body weight set-point and is ineffective in further reducing the body weight of estradiol-treated rats that are maintaining lowered body weight set-points.

During the 2-week posttreatment period, rats in the EB, DIO and CHOW condition groups that had received d-fenfluramine gained comparable amounts of body weight. Within the DIO condition group, the weight gain of rats that had received d-fenfluramine was 50% greater than that of untreated control rats in that condition group. Within the CHOW condition group, rats that had received d-fenfluramine gained 78% more weight than untreated controls. Thus rats in those condition groups that had received d-fenfluramine treatment gained substantially more weight than controls during the posttreatment period although their food intake was not significantly greater; therefore, increased weight gain during the posttreatment period occurred in the absence of significantly increased food intake. During the posttreatment period, ovariectomized rats that had been treated with d-fenfluramine continued to lose weight although at a much slower rate than during drug treatment and the total 2-week weight change was not significantly different from untreated ovariectomized rats which showed only a small weight gain during the 2-week posttreatment period.

Food efficiency [total weight gain (grams)/total kilocalories food ingested] was considerably higher in ovariectomized

estradiol-treated rats than in the other dietary or surgical condition groups. During the period of time for which food efficiency was measured, estradiol-treated rats were gaining weight at a faster rate than obese ovariectomized or unoperated Chow-fed rats and at a rate comparable to dietary obese rats with their high caloric intake. This suggests that after a period of time, the weight-reducing effects of estradiol treatment are overridden by mechanisms to conserve body weight so that continued weight loss does not occur. During this period, food efficiency was very low in ovariectomized rats that did not receive estradiol replacement. This was associated with a decreased rate of weight gain in these rats. If food efficiency had been measured in the early phases following ovariectomy when weight gain was most rapid, it might have been considerably higher in those rats that did not receive estradiol replacement.

It was not possible to estimate food efficiency in ovariectomized rats treated with d-fenfluramine because they were losing weight during the treatment and posttreatment periods. Administration of d-fenfluramine had no significant effect on the relative mean food efficiency of rats in the EB, DIO and CHOW dietary or surgical condition groups.

During the 2-week posttreatment period, the estimated food efficiency of untreated control rats in each of the four dietary or surgical condition groups was similar to their food efficiency

during the preceding 4-week period. Control rats in the estradiol-treated group had higher [weight gain/kilocalorie ingested] ratios than did untreated control rats in the other condition groups. There was no significant effect of previous d-fenfluramine treatment on food efficiency.

The similarity of food efficiency values in ovariectomized and dietary obese rats and unoperated Chow-fed rats is consistent with a recent report by Levin and colleagues (1987) in which rats maintained on a cafeteria diet had food efficiency estimates that were not different from Chow-fed controls. The failure to find a significant effect of d-fenfluramine on food efficiency is curious. During d-fenfluramine treatment, unoperated Chow-fed rats had lowered weight gain, but not significantly lowered food intake. During the posttreatment period, the rate of weight gain of rats in both the DIO and CHOW condition groups was increased relative to untreated control rats in those groups, but again food intake was not significantly affected. Thus food efficiency estimated from [weight gain/kilocalorie ingested] may not be a reliable index in these situations.

The finding that d-fenfluramine is more effective in obese than in lean animals implies that data obtained from normal weight laboratory animals may not be directly applicable to the understanding of d-fenfluramine's effects in obese humans. Other data support the notion that fenfluramine is more effective in obese

animals. (1) Data compiled from several experiments conducted in our laboratory, using diverse groups of rats (initial body weights ranging from 200-500 g), revealed a significant correlation between initial body weight and absolute weight change after ten daily injections of 5 mg d,l-fenfluramine/kg (Rowland and Carlton, 1986). Rats with initial body weights of less than 280 g gained weight during d,l-fenfluramine treatment, albeit at a slower rate than saline-injected controls, whereas rats initially weighing more than 280 g sustained an absolute weight loss. (2) Previous studies in our own and other laboratories (e.g. Carlton and Rowland, 1986; Fantino et al., 1986; Stunkard and Goodall, 1986) have shown that d-fenfluramine is more effective in reducing food intake and body weight of rats with diet-induced obesity. (3) In obese humans, both d,l-fenfluramine (Innes et al., 1977) and, to a greater extent, d-fenfluramine (Pleas, 1986) therapy can produce sustained weight loss.

Fenfluramine is effective in producing weight loss in obese humans (Burland, 1975; Innes et al., 1977; Munro and Ford, 1978). At a dose of 60-120 mg/day (vis. 1-2 mg/kg/day), d,l-fenfluramine produces an average weight loss of approximately 10 kg over a period of 6 months (Innes et al., 1977; Stunkard, 1981). Body weight is maintained at a lowered level until drug therapy is discontinued. Weight is regained within about 12 months of termination of drug treatment (Douglas et al., 1983; Stunkard,

1981). With d-fenfluramine, at a dose of 30-60 mg/day, weight loss maybe sustained for much longer periods of time (up to 2 years or more) (Pleas, 1986). Fenfluramine is lipid soluble and accumulates in adipose tissue so that steady plasma levels are attained within several days of treatment initiation (Rowland and Carlton, 1986).

In normal weight laboratory animals fed laboratory Chow on a 4 hour/day feeding schedule, a dosage of 5 mg d,l-fenfluramine/kg acutely suppresses food intake by approximately 80%. Thereafter, anorectic tolerance develops rapidly and food intake returns to baseline levels within 5-7 days of chronic administration (Antelman et al., 1981; Ghosh and Parvathy, 1976; Goudie et al., 1974; Rowland et al., 1983). A similar tolerance curve is obtained in paradigms that do not involve food deprivation such as "dessert" tests (Carlton and Rowland, 1984) and tail pressure-induced eating (Antelman et al., 1981). Tolerance to d-fenfluramine resembles that seen with the racemate; however, the d-isomer is much more potent and tolerance may be delayed or incomplete with chronic injections of 5 mg/kg or greater (Rowland and Carlton, 1986).

The effects of d- and d,l-fenfluramine on the body weights of normal weight animals parallel its anorectic effects: there is an initial dose-dependent weight loss followed by weight gain at a trajectory parallel to controls (eg. Duhault et al., 1979; Brindley et al., 1985). In obese animals, anorexia may be prolonged

and weight loss sustained over longer periods of time with either d,l- or d-fenfluramine (Carlton and Rowland, 1986; Rowland and Carlton, 1986).

Several lines of evidence suggest that fenfluramine produces sustained weight loss in obese humans and laboratory animals by antiobesity actions that may be independent of appetite suppression: (a) the maintenance of weight loss until termination of drug therapy, followed by rebound weight gain seen clinically; (b) sustained weight loss accompanied by anorectic tolerance found under certain experimental conditions; (c) increased drug efficacy in obese versus lean animals and (d) enhanced loss of body fat in obese women.

The increased drug efficacy in obese animals relative to lean controls has been interpreted in terms of a set-point model (Stunkard and Goodall, 1986). According to Stunkard's theory, fenfluramine acts to lower the level at which body weight is regulated resulting in decreased food intake and concomitant weight loss until the new level is reached.

Levitsky et al. (1981) proposed that the apparent anorectic tolerance to daily injections of d,l-fenfluramine that develops in laboratory animals is due to increasing deprivation and weight loss rather than a true biological drug tolerance. This conclusion was based on a study in which those authors reported that rats with experimentally induced body weight reductions showed no

anorexia compared to normal-weight control rats when treated acutely with a high dosage of d,l-fenfluramine (20 mg/kg). The interpretation of those results has not gone unchallenged (Carlton and Rowland, 1985, Rowland and Carlton, 1986); however, that study, along with the clinically observed weight "rebound" that often occurs upon termination of d,l-fenfluramine treatment, formed the basis for Stunkard's set-point model. This model has been applied to the actions of both d- and d,l-fenfluramine.

There are two aspects of the set-point model that are relevant here: one concerning anorexia and weight loss and the other concerning tolerance to these effects. According to Stunkard's set-point model, fenfluramine initially acts to lower the level at which body weight is regulated. This results in decreased food intake and weight loss until the new level is reached. The new level is maintained, and presumably defended, as long as drug treatment is continued.

The set-point model has also been used to explain the apparent development of tolerance to d,l-fenfluramine-induced anorexia that occurs in most animal studies (Levitsky et al., 1981; Stunkard, 1981). Accordingly it was suggested that changes in nutritional status resulting from drug-induced food deprivation and/or reduced body weight increase motivation to eat and hence produce an apparent anorectic tolerance. This line of reasoning has been extended to include clinical observations. Thus it was

suggested that obese humans do not develop tolerance to d,l-fenfluramine-induced anorexia and weight loss as quickly as do experimental animals because their nutritional reserves are large enough that the relevant period of food deprivation does not lead to reduced anorectic potency.

We have reported that pretreatment food deprivation and weight loss does not prevent acute anorexia in normal weight rats treated with daily injections of 5 mg d,l-fenfluramine/kg, suggesting that anorectic tolerance in this paradigm is not an artifact of weight loss (Carlton and Rowland, 1985). This is in apparent opposition to the hypothesis that fenfluramine acts primarily to lower body weight set-point. Moreover, tolerance to fenfluramine anorexia develops in paradigms that do not involve deprivation or weight loss such as eating in response to tail pinch (Antelman et al., 1979), muscimol-stimulated eating (Borsini et al., 1983) and "dessert" tests (Carlton and Rowland, 1984). Clearly, a simple model of set-point regulation cannot be universally applied to explain all of the observed effects of fenfluramine.

The preceding studies have used alteration in food intake as an indicator of changes in body weight set-point. Inferences about set-point derived from changes in body weight are tautological and so, even less convincing. Fantino et al. (1986) recently have proposed a more powerful model for understanding

body weight regulation and set-point in rats based on hoarding behavior. Rats normally do not hoard food when fed ad libitum, but begin to do so when they lose weight as a result of food deprivation. In Chow-fed rats, hoarding begins with a weight loss of less than 10 g and the amount of food hoarded is proportional to weight loss. A 30% loss in body weight results in intense hoarding behavior that decreases in proportion to restoration of body weight. According to this model the body weight set-point is the weight at which the amount hoarded reaches zero on the line of regression for amount hoarded versus body weight.

This model has been used to study dietary obesity and the effects of d-fenfluramine (Fantino et al., 1986). Hoarding behavior in Chow-fed rats treated with d-fenfluramine did not begin until the animals lost 60 g, thus supporting the notion that d-fenfluramine lowers body weight set-point. Rats fed a cafeteria diet did not begin hoarding until they had lost 50 g, suggesting that body weight was maintained above the set-point level. The effects of a palatable diet sustained eating above the set-point regulated level. Dextrofenfluramine further increased the amount of weight loss necessary to invoke hoarding behavior in rats with diet-induced obesity to 120 g.

The increased efficacy of d-fenfluramine in ovariectomized obese rats and the lack of effect of the drug in ovariectomized estradiol-treated rats also fits within a set-point model.

Accordingly estradiol-treated rats are maintaining lowered body weights relative to ovariectomized or unoperated rats; therefore, subsequent treatment with d-fenfluramine does not further decrease body weight. Conversely ovariectomized rats that do not receive estradiol are maintaining their body weights at an elevated level and hence are more sensitive to the drug's effects. Similar arguments are among the most common in set-point theory. This provides an easy explanation for the increased efficacy of d-fenfluramine in obese animals, but contributes very little to the understanding of body weight regulation or of the actions of drugs affecting appetite and body weight. For example, if an obese animal is maintaining its body weight at an elevated set-point and a normal animal is maintaining its body weight at its set-point, why is one more easily changed than the other?

An alternative approach is to examine the effects of d-fenfluramine and of estrogen on metabolic processes that may be involved in energy regulation. There are a number of similarities between the reported actions of estrogen and d-fenfluramine. It may be that both cause a similar physiological response that is not additive and that one of them is sufficient for the purpose, or that the two work differently but achieve a similar result.

The effects of d-fenfluramine in obese animals does seem to fit a set-point model; however, this is not a complete explanation and it does not encompass all the data. Such a model is of little

heuristic value in explaining the effects of d-fenfluramine or other anorectic drugs on food intake and body weight. Rather than using hypothetical constructs to explain physiological phenomena, a much more productive approach is to examine possible mechanisms by which this can occur.

Effects of d-Fenfluramine on Adipocyte Size

A decrease in lipid storage associated with weight loss may be reflected in a decrease in adipocyte size in one or more fat depots. To determine if weight loss associated with chronic d-fenfluramine treatment is a result of decreased lipid storage, the diameters of white and brown adipocytes were measured.

The mean diameters of inguinal white adipocytes were similar in untreated control rats from the OVX (43 μ m), DIO (44 μ m) and CHOW groups (43 μ m), but the mean adipocyte diameter of rats from the EB group, was 22% smaller than that of the OVX group. This indicates that the increased adiposity of obese ovariectomized and dietary obese rats in this study reflects adipocyte hyperplasia since there is no evident hypertrophy.

Administration of d-fenfluramine for 4 weeks significantly reduced inguinal adipocyte diameters in dietary obese and unoperated Chow-fed rats and, to a lesser extent, in ovariectomized rats, but had no effect on the diameters of inguinal adipocytes of estradiol-treated rats. The effect of d-fenfluramine treatment was to reduce the mean inguinal adipocyte

diameters of the dietary obese and unoperated Chow-fed rats by approximately 45%. Fenfluramine had no effect on the mean adipocyte diameter of the estradiol-treated rats; therefore, after 4 weeks of chronic administration, the mean adipocyte diameters of dietary obese and unoperated Chow-fed rats were smaller than adipocyte diameter of the estradiol-treated rats. Since d-fenfluramine was differentially effective in reducing mean inguinal adipocyte diameter in the different dietary or surgical condition groups, there was a significant interaction between the group condition and treatment variables.

By 2 weeks after the termination of d-fenfluramine treatment, the mean inguinal adipocyte diameters of ovariectomized rats had returned to untreated control levels. Mean adipocyte diameters of dietary obese and unoperated Chow-fed rats that had received d-fenfluramine had partially recovered within 2 weeks, but remained below control levels.

Brown adipocyte diameters were not significantly different among the four dietary or surgical condition groups and d-fenfluramine treatment had no effect on the sizes of these adipocytes. Total interscapular brown adipose tissue (IBAT) pad weights were not measured in these rats; however, during the tissue dissections, it was obvious that IBAT pads were much larger in ovariectomized and dietary obese rats and were very small in estradiol-treated rats compared to unoperated Chow-fed rats. This

may be related to the long-term exposure to the procedures used to induce obesity. With short-term feeding of a cafeteria diet, interscapular brown adipocytes increase in size (Tulp, 1981). With relatively long-term feeding of a similar diet, cell size may decrease, but cell number increases (Triscari et al., 1985; Tulp, 1981).

The relative increase in IBAT weight that occurs subsequent to ovariectomy or diet-induced obesity has been associated with increased thermogenesis and metabolic activity (Himms-Hagen et al., 1981). Alternatively it has been suggested that the increased IBAT weight is the result of excess lipid storage in this tissue (Hervey and Tobin, 1983). The failure of d-fenfluramine to reduce brown adipocyte diameters in cases where it reduced white adipocyte diameters and body weight does not distinguish between the two possibilities; however, it does imply that if the increased IBAT weight is due primarily to increased lipid storage, that this store is less susceptible to the actions of d-fenfluramine on lipid storage.

A part of the variability in studies on diet-induced obesity may be due to differential changes in size and number of adipocytes with different ages and lengths of exposure to special diets. This is an important issue with regard to both white and brown adipose cellularity. Removal of a palatable diet from obese rats can result in a temporary increase in thermogenic capacity

(Levin et al., 1983). Those rats may then become leaner (Stephens, 1980) and show resistance to future development of obesity (Brooks et al., 1981). After 9 weeks or more of overfeeding, hyperplasia may develop (Faust et al., 1978; Obst et al., 1981) and animals may subsequently maintain relatively higher body weights (Rolls et al., 1980). Metabolic activity of adipocytes may change as cells change size when reported as activity per cell (Brunzell, 1979), or as a function of surface area (Brunzell and Greenwood, 1983).

Blundell and Hill (1985) have reported a time-dependent difference in the effects of d-fenfluramine administered in drinking water on food intake and weight loss of animals on a cafeteria diet. During the dynamic phase of weight gain, d-fenfluramine was equally effective in cafeteria- and Chow-fed rats; however, after 76 days on the cafeteria diet, weight gain had reached a plateau and d-fenfluramine was significantly more effective in obese rats than in Chow-fed controls.

The time course for development of adipocyte hyperplasia appears to be of critical importance in assessing the long-term effects of overfeeding and weight changes subsequent to changes in diet. Overfeeding for a period of time that is sufficient to allow hyperplasia to develop appears to have long-lasting or permanent effects on body weight. In humans or experimental animals with increased adipocyte number, dietary restriction or

anorectic drug treatment may decrease the amount of lipid stored in existing adipocytes; however, when storage is reduced to a given level, further reductions become increasingly difficult. When dietary restriction is eased, or drug treatment terminated, adipocytes will not have decreased in number and may simply refill.

Effects of d-Fenfluramine on Brain Serotonin (5-HT)
and Dopamine (DA)

The actions of fenfluramine on brain 5-HT have been studied extensively. Until recently, the majority of behavioral studies have assumed that observed drug effects were primarily, if not exclusively, mediated by brain 5-HT. Blundell (1977, 1979) has published reviews on the involvement of brain 5-HT in the regulation of feeding behavior. The precise nature of this role and the pathways involved have not been defined; however, it is believed to be generally inhibitory.

Leibowitz and Shor-Posner (1986) have reported a dose-dependent inhibition of food intake after injection of 5-HT or norfenfluramine into the paraventricular nucleus (PVN) of the hypothalamus. Moreover, the observed effects on the microstructure of feeding were similar to the effects of peripherally administered fenfluramine (vis. a slowing of the rate of feeding and early termination of meals). It should be noted that although these results seem to suggest a central mode of action, d,l-fenfluramine has a low potency when administered centrally by

intracerebroventricular (icv.) infusion (Rowland and Carlton, 1986) or by direct injection into the hypothalamus arguing against a primary central action (Davies et al., 1983).

Fenfluramine competitively inhibits [^3H]5-HT reuptake by brain synaptosomes and stimulates its release from vesicular storage sites (Fuxe et al., 1975; Garattini et al., 1979). The d- and l-enantiomers stimulate release with approximately equal potency (Duhault and Verdavainne, 1967; Garattini et al., 1975; Ghezzi et al., 1973). Dextrofenfluramine is approximately 10-times more potent in inhibiting uptake than is the l-isomer (Garattini et al., 1979; Mennini et al., 1985). Anorectic potency is thus correlated with inhibition of uptake (Mennini et al., 1985); however, inhibition of 5-HT reuptake alone may not be sufficient to produce anorexia (Samanin et al., 1980a) or may do so by changing meal frequency rather than meal size (Burton et al., 1981).

Chronic administration of moderate dosages of d,l-fenfluramine (<10 mg/kg/day) and acute administration of higher doses of d- or d,l-fenfluramine are associated with decreased brain levels of 5-HT and 5-HIAA in rats (Duhault and Verdavainne, 1967; Duhault et al., 1979, 1981; Garattini et al., 1979). Depletion of 5-HT begins within the first 1-2 hours of drug administration and is followed by decreases in 5-HIAA (Duhault and Verdavainne, 1967; Duhault et al., 1980; Fuller et al., 1978). This suggests that

5-HT is depleted due to increased release without a compensatory increase in synthesis.

The rate of 5-HT synthesis in brain is normally limited by the availability of the precursor amino acid tryptophan. Acutely administered d,l-fenfluramine has been reported to increase (Tagliamonte et al., 1971) or have no effect (Costa et al., 1971) on brain levels of tryptophan; however, the rate of 5-HT synthesis measured by accumulation of the immediate precursor 5-hydroxytryptophan (5-HTP) following inhibition of 5-HTP decarboxylase is decreased by acute fenfluramine.

Decreases in synthesis are found as early as 2 hours after administration of 10 mg/kg d,l-fenfluramine (Rowland and Carlton, 1986) and may last up to 8 days after a single dose of 15 mg/kg d,l-fenfluramine (Clineschmidt et al., 1978). The ratio of 5-HIAA/5-HT is initially increased (Fuller et al., 1978; Oroscio et al., 1984) which further suggests increased neuronal activity; however, it is not clear whether this increased activity is sustained throughout the period of drug administration.

Decreases in whole brain 5-HT produced by high doses of d,l-fenfluramine are still evident at one month after acute administration (Clineschmidt et al., 1978) although most of the drug is cleared within 24 hours (Garattini et al., 1979). This long-lasting depletion may be due to a neurotoxic action on the nerve terminal (Fuller, 1981). The binding of [¹⁴C]fenfluramine

to brain tissue preparations (Belin et al., 1976; Duhault et al., 1980) implies that fenfluramine can bind to 5-HT receptors. Thus, the fenfluramine-stimulated release of 5-HT may be dependent upon binding of the drug to presynaptic receptors (Rowland and Carlton, 1986). Depletion of brain 5-HT can be prevented by prior administration of drugs that block 5-HT reuptake (Clineschmidt et al., 1978; Fuxe et al., 1975) and so apparently requires the uptake of fenfluramine into the nerve terminal.

Both fenfluramine and norfenfluramine inhibit the *in vitro* binding of [³H]5-HT to rat brain membranes. The efficacy of the *l*-enantiomers ($IC_{50} = 2 \text{ } \mu\text{mol}$) is double that of *d*-norfenfluramine ($IC_{50} = 4 \text{ } \mu\text{mol}$) and more than 3-times greater than that of *d*-fenfluramine ($IC_{50} = 7 \text{ } \mu\text{mol}$) (Mennini et al., 1985).

In the present experiment, the effects of continuous administration of 3 mg *d*-fenfluramine/kg/day via osmotic minipump on concentrations of 5-HT and 5-HIAA and the ratio of 5-HIAA/5-HT in rat telencephalon and hypothalamus were measured. It was hypothesized that depletions in brain 5-HT reported in previous animal studies might be the result of administering the drug by injections that cause an acute peak in the drug concentration in brain followed by a rapid decline.

Unlike administration of *d*- or *d,l*-fenfluramine by intermittent injections, continuous delivery by osmotic minipump for 4 weeks at a dosage of 3 mg/kg/day had no significant effects on the

concentrations of 5-HT or 5-HIAA or on the ratio of 5-HIAA/5-HT in the telencephalon. There was no effect of d-fenfluramine treatment on telencephalic 5-HT that was evident at 2 weeks after the termination of treatment.

There were significant increases in the concentrations of 5-HT and 5-HIAA in the hypothalamus, but the ratio of was not significantly affected. Two weeks after the termination of d-fenfluramine treatment, the concentration of 5-HT in the hypothalamus had returned to control levels.

These results strongly support the hypothesis that depletions of brain 5-HT are an artifact of the mode of administration and the metabolic profile of d-fenfluramine in rats. Using a treatment regimen designed to more closely resemble clinical regimens, chronic administration of d-fenfluramine, at a dose that produced significant reductions in body weight, did not deplete brain 5-HT. The increase in hypothalamic 5-HT and 5-HIAA concentrations after 4 weeks of d-fenfluramine administration was not expected. Perhaps with steady, relatively low concentrations of d-fenfluramine, increased release is accompanied by increased synthesis. The lack of change in the ratio of 5-HIAA/5-HT, insofar as this reflects 5-HT turnover, implies that any changes in release and reuptake are accompanied by changes in synthesis and metabolism so that actual turnover is not changed significantly. The effects of d-fenfluramine on brain 5-HT release, reuptake or binding were not

directly measured in the present experiment; therefore, it is not possible to draw definitive conclusions on the effect of this treatment regimen on brain 5-HT activity. It is clear however, that this treatment regimen did not deplete brain 5-HT.

The finding that continuously administered d-fenfluramine sustained suppression of food intake and body weight, but did not deplete brain 5-HT has important clinical implications. One of the major arguments against the use of fenfluramine in humans has been the possibility of damage to brain 5-HT neurons. In showing that a treatment regimen designed to closely resemble a clinical therapy regimen does not deplete brain 5-HT, a number of issues have been raised that seriously question the applicability of many previous neurochemical studies to the clinical situation.

This also may explain some of the disparity between animal and clinical studies. If indeed tolerance to fenfluramine is due largely to depletions of brain 5-HT, the failure of human subjects, as well as animals in this study, to develop complete tolerance may be explained by the fact that brain 5-HT is not depleted.

This finding also has serious implications for studies on the peripheral actions of d-fenfluramine, many of which are thought to be mediated by 5-HT. Changes seen in these parameters with chronic, compared to acute, fenfluramine may be the result of 5-HT depletion by high doses or concentrations that would not occur clinically.

Continued research on the effects of fenfluramine has yielded results that are not readily explainable in terms of a singular brain 5-HT mechanism. In the last 2-3 years, these inconsistencies have become increasingly difficult to ignore.

Changes in the number of [^3H]-5HT binding sites (B_{max}) have been reported following procedures that either increase or decrease central 5-HT availability (Hamon et al., 1980). A decrease in the number of [^3H]-5-HT binding sites in rat frontal cortex has been reported following 28 days, but not 14 days, of chronic d-fenfluramine administration (2.5 mg/kg twice daily) (Samanin et al., 1980b). This was originally interpreted as evidence that subsensitivity of 5-HT receptors may underlie the development of anorectic tolerance; however, the discrepancy between the time courses for the reported biochemical changes and the development of anorectic tolerance suggests that these phenomena are not functionally related. We have been unable to confirm those observations and have not found consistent changes in binding of [^3H]-5-HT (putative S_1 receptors) or [^3H]-spiroperidol (putative S_2 receptors) to membrane preparations from various brain regions following chronic d,l-fenfluramine (Rowland et al., 1983).

If peripherally administered fenfluramine exerts its anorectic effects via enhanced central 5-HT transmission, one would expect that injection directly into brain tissue or cerebrospinal

fluid would be similarly effective. Broekkamp et al. (1975) first reported that d,l-norfenfluramine administered into the stria terminalis reduced food intake of food deprived rats, but equal doses of d,l-fenfluramine were considerably less effective. Kruk (1973) showed that icv. injection of d,l-norfenfluramine was also effective; however, those studies used high drug doses to produce modest effects on food intake (Davies et al., 1983, Rowland and Carlton, 1986).

The 5-HT receptor blocker metergoline reliably reverses d,l-fenfluramine-induced anorexia when both are administered peripherally (Clineschmidt et al., 1974); however, peripherally administered metergoline does not block anorexia produced by centrally administered d,l-fenfluramine (Rowland and Carlton, 1986). This strongly suggests that peripherally administered fenfluramine does not act exclusively via central 5-HT. Anorexia produced by centrally administered d,l-fenfluramine or norfenfluramine can be partially overcome by tail pressure stress whereas the anorexia produced by peripheral administration is not affected (Antelman et al., 1979, 1981) again suggesting disparate mechanisms.

Another way to examine the role of central 5-HT in mediating fenfluramine's actions is to deplete brain 5-HT prior to drug administration. If the primary action of fenfluramine is via its effects on release and uptake of 5-HT at presynaptic terminals,

damage to these terminals resulting from electrolytic or neurotoxic lesions would be expected to reduce the efficacy of fenfluramine. Rowland and Carlton (1986) reviewed the effects of various techniques for producing brain 5-HT depletions on d,l-fenfluramine-induced anorexia in rats. A table listing the results (p. 47) showed that anorexia was attenuated in four cases, enhanced in four cases and not affected in three cases (two of the studies reported results on two groups). We had previously reported that substantial depletions of brain 5-HT induced by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) resulted in a slight increase in the efficacy of 2 and 5 mg, and a decrease in the efficacy of 10 mg d,l-fenfluramine/kg (Carlton and Rowland, 1984).

Concentrations of DA and DOPAC in the telencephalon and hypothalamus and the ratios of DOPAC/DA in those regions were not affected significantly by the treatment regimen used in the present study. Injections of d,l-fenfluramine have been reported to increase concentrations of the extraneuronal DA metabolite homovanillic acid (HVA) in the striatum that may reflect increased DA release. Tolerance to this effect is not apparent, at least within the time period for anorectic tolerance (Jori and Bernardi, 1972; Jori and Dolfini, 1977). The l-isomer is considerably more potent in stimulating DA activity; however, the d-isomer is also effective in this regard (Jori and Dolfini, 1974; Garattini et al., 1975). The effect of d,l-fenfluramine on striatal DA

turnover is blocked by pretreatment with DA receptor antagonists and therefore appears to be a result of receptor blockade (Crunelli et al., 1980). Although DA antagonists such as neuroleptics can reduce food intake (eg. Rowland and Engle, 1977), this is not thought to be a primary mechanism of fenfluramine anorexia. The possibility of interactions among DA, 5-HT and opioid systems in the central control of food intake and in the actions of fenfluramine, cannot be dismissed however. Opioid and DA systems have been implicated in stress-induced eating and may also be important in mediating the rewarding effects of food.

Peripheral Actions of d-Fenfluramine

The present study confirms the hypothesis that d-fenfluramine is more effective in producing sustained weight loss in animals with a higher percentage body fat. Evidence has been presented that argues against a singular brain 5-HT mechanism as the primary mediator of d-fenfluramine's actions on food intake and body weight. Furthermore, the actions of d-fenfluramine on food intake and body weight imply that the compound has antiobesity actions other than appetite suppression.

In the present experiment, prolonged anorexia and decreased weight gain occurred in rats made obese by feeding a palatable and varied diet. Enhanced and prolonged weight loss occurred in obese ovariectomized rats whereas food intakes and body weights of lean ovariectomized estradiol-treated rats were not affected by d-fenfluramine treatment, even acutely.

Peripheral actions of fenfluramine may be relevant to its antiobesity actions and may also help explain possible differences in drug efficacy in lean and obese animals. Effects of d,l-fenfluramine on carbohydrate metabolism are found at drug concentrations that are clinically relevant. These include (a) increased glucose uptake (Butterfield and Whichelow, 1968) and production of lactate (Kirby and Turner, 1976) suggesting that relatively inefficient anaerobic glucose metabolism is stimulated; (b) increased insulin receptor sensitivity (Verdy et al., 1983) and (c) improved glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970).

Effects of d- and d,l-fenfluramine on lipid metabolism have been studied primarily in vitro using high drug concentrations; therefore, the relevance of these actions to anorexia and weight loss is questionable. Previously reported effects include (a) a decrease in total plasma lipids (Pawan, 1970); (b) a decrease in the meal-related rise in plasma triglycerides (Bizzi et al., 1973; Curtis-Prior et al., 1980; Garattini et al., 1975); (c) an acute increase in circulating fatty acids (FFA), ketones and glycerol (Pawan, 1970) and (d) inhibition of lipogenesis in vitro and in vivo (Wilson and Galton, 1971; Comai et al., 1978) that may be related to lower insulin binding in adipocytes (Harrison et al., 1975). Nicolaidis and Even (1986) have reported a prolonged stimulation of lipolysis and enhanced utilization of lipid substrates using d-fenfluramine (7.5 mg/kg).

Acutely, d,l-fenfluramine stimulates corticosterone release (Fuller et al., 1981; McElroy et al., 1984; Schettini et al., 1979) and has mild sympathomimetic actions including increased plasma norepinephrine (NE) (Calderini et al., 1975; Lake et al., 1979); inhibition of insulin release and stimulation of glucagon release (Barseghian et al., 1983). These effects may be mediated in part by central or peripheral 5-HT: exogenously administered 5-HT stimulates sympathetic nervous system activity (Stajarne and Schapiro, 1959; de Groat and Volle, 1966) and administration of 5-HTP provokes release of insulin and glucagon in vivo (Jacoby and Bryce, 1978). Previous reports imply that acute and chronic drug effects may differ. For example, chronic administration of a high dose of d-fenfluramine decreases the stress-evoked rise in concentrations of corticosterone and catecholamines (Brindley et al., 1985); furthermore, decreases in plasma NE and other indicators of sympathetic nervous system activity are found with chronically administered d,l-fenfluramine (Lake et al., 1979; Rothwell et al., 1982).

Large doses of d,l-fenfluramine, chronically administered, increase GDP-binding to brown adipose tissue (BAT) mitochondria (Bray and Lupien, 1984; Lupien and Bray, 1985) which presumably reflects increased thermogenic activity. This effect is not seen with lower doses and others have reported that d-fenfluramine has no effect on resting metabolic rate although the energy cost of

locomotor activity may be increased (Nicolaidis and Even, 1986) as well as the thermic effect of food (Levitsky, 1986).

The relevance of any of the central or peripheral actions of fenfluramine to appetite suppression and weight loss is not completely clear. When various actions are viewed in isolation or when effects of single doses and high concentrations of drug are extrapolated to explain clinical actions, the results may seem confusing and contradictory. Only by putting together the series of actions as they occur within a relevant treatment model is it possible to determine the exact nature of the factors and interactions important to appetite regulation and weight loss.

As discussed previously, a productive approach to understanding the antiobesity effects of d-fenfluramine is to examine possible drug actions that may affect body weight either in relation to decreased food intake or in apparent absence of major effects on food intake. Several of the possible antiobesity actions of d-fenfluramine were examined in this regard.

NPPase activity. The activity of K^+ -nitrophenylphosphatase (NPPase) is an index of the activity of the enzyme $(Na^+-K^+)ATPase$ and hence reflects cellular activity (Swann, 1984a, 1984b). The activity of NPPase was measured in interscapular brown adipose tissue (IBAT) and in gastrocnemius muscle of d-fenfluramine-treated and untreated control rats from each of the four dietary or surgical conditions. The tissues were selected because of

previously reported effects of d,l-fenfluramine on the thermogenic efficiency of brown adipose tissue and effects of d-fenfluramine on the cost of muscular activity. It was hypothesized that if d-fenfluramine, at a clinically relevant dose, acts to decrease body weight by increasing energy burning in brown adipose tissue or by increasing the cost of muscular activity, these actions may be reflected in changes in the activity of NPPase in those tissues. It was further hypothesized that differences in enzyme activity may be associated with obesity induced by diet or ovariectomy and that NPPase activity in obese rats might respond differentially to d-fenfluramine treatment thus offering an explanation for differences in the drug's effects on body weight and food intake in the various dietary or surgical condition groups.

The activity of NPPase in BAT of ovariectomized rats was low compared to control rats in the other condition groups and was less than 25% of control values from unoperated Chow-fed rats. Administration of d-fenfluramine increased NPPase activity in BAT of ovariectomized rats to a level comparable to the other groups. Fenfluramine also increased NPPase activity in unoperated Chow-fed rats by 45% but did not significantly affect the activity of this enzyme in dietary obese or estradiol-treated rats.

By the end of the 2-week drug-free period, d-fenfluramine-induced increases in NPPase activity in BAT were completely or partially reversed. In ovariectomized rats, the increased NPPase

activity associated with d-fenfluramine administration was not reversed completely within 2 weeks, but was significantly lower than NPPase activity measured at the termination of drug treatment. There was a significant interaction between the treatment and dietary or surgical condition group variables because d-fenfluramine treatment increased NPPase activity in rats from the ovariectomized and unoperated Chow-fed groups, but not in dietary obese and estradiol-treated rats.

The changes in activity of NPPase in BAT were consistent with changes in body weight in all except dietary obese rats. The activity of NPPase was low in ovariectomized rats and was substantially increased by chronic d-fenfluramine treatment. The low NPPase activity in untreated ovariectomized rats was associated with obesity that was not maintained by increased food intake. Administration of d-fenfluramine caused a substantial weight loss and increased NPPase activity. Fenfluramine treatment did not increase NPPase activity BAT of dietary obese rats. Estradiol administration also increased NPPase activity in BAT of ovariectomized rats, but d-fenfluramine treatment did not further increase NPPase activity in estradiol-treated rats and was not effective in causing weight loss in those rats.

Long-term feeding of a cafeteria diet may cause changes in NPPase activity in BAT so that it is more difficult to affect enzyme activity. Thus d-fenfluramine increased NPPase activity in

Chow-fed, normal weight rats and ovariectomized rats with low control levels of NPPase, but was not effective in increasing NPPase activity in rats that may have alterations in enzyme activity caused by prolonged stimulation of NPPase at some point. Long-term stimulation of this indicator of ATPase activity may lead to changes in beta-adrenergic activity or in sympathetic nervous system activity and subsequent desensitization to stimulation by d-fenfluramine. Levin et al. (1983) found increased NE turnover in BAT of rats fed a cafeteria diet for 7 days but after 3 months on this diet, plasma NE levels were lower than Chow-fed controls and NE turnover in organs was decreased. In that study, NE-stimulated lipolysis was decreased in BAT and cold-induced thermogenesis was impaired, both of which suggest defects in sympathetic postsynaptic receptors.

The low levels of NPPase activity in ovariectomized rats are consistent with reports of lowered thermogenic activity in ovariectomized rats that is dependent upon sympathetic nervous system activity and is reversed by administration of estradiol benzoate (Bartness and Wade, 1984). The d-fenfluramine-stimulated increase in BAT thermogenesis in ovariectomized and unoperated Chow-fed rats may be related to increased sympathetic activity since acutely administered fenfluramine has mild sympathomimetic effects (Lake et al., 1979) that may be 5-HT mediated (Stajarne and Schapiro, 1959). In the current study, d-fenfluramine

administration did not brain deplete 5-HT, therefore the chronic treatment effects seen here may be similar to acute effects observed with higher doses.

The activity of NPPase in gastrocnemius muscle was decreased in untreated ovariectomized rats to approximately the same extent as enzyme activity in BAT (<25% of CHOW control value). The NPPase activity of dietary obese rats was also reduced, although to a lesser extent than in ovariectomized rats (55% of CHOW control value). Activity of muscle NPPase in control estradiol-treated rats was increased by approximately 39% over control values from the CHOW group. This dramatic difference between muscle NPPase activity of obese ovariectomized and estradiol-treated rats may be related to differences in activity levels between the two groups. Ovariectomized rats have decreased activity levels compared to unoperated female rats and administration of estradiol increases activity (Wade and Gray, 1979). The markedly low levels of NPPase activity in both BAT and muscle of ovariectomized rats is a probable explanation, at least in part, for the obesity in these rats in the absence of increased food intake.

Administration of d-fenfluramine for 4 weeks increased the activity of muscle NPPase in ovariectomized rats by approximately 47%, but the increase was not sufficient to raise enzyme activity in these rats to control levels of unoperated Chow-fed rats.

Administration of d-fenfluramine had no significant effect on muscle NPPase activity in rats from the other dietary or surgical condition groups compared to untreated controls in those groups, thus enzyme activity in d-fenfluramine-treated dietary obese rats remained below the level of activity in unoperated Chow-fed control rats. Perhaps the pronounced effects of d-fenfluramine on NPPase activity in ovariectomized rats and the relative lack of effectiveness in dietary obese rats is one of the reasons for the greater efficacy of d-fenfluramine in producing weight loss in ovariectomized rats compared to the dietary obese and other groups although the hypophagic effects were more obvious in dietary obese rats.

Even and Nicolaidis (1986) have reported that acutely administered d-fenfluramine decreases the efficiency of locomotor activity in rats but has no effect on basal metabolism. Rowland (1986) found no difference in the effects of d,l-fenfluramine on body weights of exercising versus sedentary hamsters. The failure of d-fenfluramine to increase gastrocnemius muscle NPPase activity in muscle of any but ovariectomized rats does not appear to be consistent with the increased cost of muscular activity in normal weight rats (Nicolaidis and Even, 1986); however, those studies used a higher drug dose (7.5 mg/kg). This further implies that ovariectomized rats are more sensitive to the effects of d-fenfluramine. At the end of the 2-week drug-free period, NPPase

activity in gastrocnemius muscle of ovariectomized rats that had been treated with d-fenfluramine had returned to control levels.

Experimental evidence (eg. Rothwell and Stock, 1979a; 1979b), along with clinically observed variations in efficiency of metabolism, suggest that, in some situations, increased caloric intake is accompanied by increased energy expenditure and an increase in diet-induced thermogenesis (Danforth, 1981; Landsberg and Young, 1981; Rothwell and Stock, 1979b). In experimental animals, this has been attributed to increased metabolic activity in brown adipose tissue (Rothwell and Stock, 1979a; Stirling and Stock, 1960). Cafeteria feeding for a period of 2 weeks has been associated with hypertrophy of IBAT (Armitage et al., 1983; Himms-Hagen et al., 1981; Rothwell and Stock, 1979b) and unmasking of GDP-binding sites in BAT mitochondria (Himms-Hagen et al., 1981). Analogous changes are seen with cold exposure (Armitage et al., 1983) and with chronic administration of NE (Desautels and Himms-Hagen, 1979; Himms-Hagen et al., 1981). The diet-induced increase in IBAT weight persists with return to Chow feeding (Tulp, 1981) whereas IBAT size returns to normal upon termination of cold exposure (Himms-Hagen et al., 1972).

Sympathetic activity is critical in the regulation of thermogenic activity in BAT and other tissues (Depocas et al., 1978; Desautels and Himms-Hagen, 1979; Fain et al., 1973; Foster and Frydman, 1978; Horowitz, 1973; Seydoux et al., 1977). In both

brown and white adipose tissue, sympathetic activity at beta-adrenergic receptors stimulates lipolysis providing FFA for subsequent beta-oxidation. In BAT, FFA may also uncouple mitochondrial oxidative phosphorylation (Fain et al., 1973). Increased NE turnover reported in cafeteria-fed rats may be a mechanism by which thermogenesis is stimulated (Levin et al., 1983).

Swann (1984a) reported diet-induced changes in activity of the enzyme $(\text{Na}^+-\text{K}^+)\text{ATPase}$ in BAT and muscle that appeared to be regulated by beta-adrenergic receptors. Cafeteria feeding increased ATPase activity and that activity remained elevated with return to regular diet. The rats in that experiment increased their caloric intake by 80% but did not gain weight relative to controls. Food deprivation resulted in decreased ATPase activity that persisted upon refeeding. During the period of refeeding, those animals gained weight approximately 3-times faster than nondeprived controls. Insofar as ATPase activity is an indicator of thermogenic activity in BAT, that experiment suggested that overfeeding associated with increased activity in BAT does not produce weight gain, but food restriction produces decreases in BAT activity that increase the efficiency of subsequent weight gain.

Other evidence contradicts the notion that BAT thermogenesis is crucial to changes in energy metabolism with variation in diet. In carefully controlled metabolic studies, Armitage et al.

(1983) were able to account for increased energy expenditure during cafeteria feeding entirely by adding the increased energy costs of digestion, fat synthesis and increased body size. Triscari et al. (1985) reported hyperinsulinemia and greatly increased efficiency of energy utilization in rats with diet-induced obesity. Removal of IBAT, or section of the sympathetic fibers innervating this tissue, increase the deposition of lipids in white adipose tissue during sucrose feeding (Granneman and Campbell, 1984). Similar results have been interpreted as evidence in favor of an "energy burning" role for BAT; however, in the experiments conducted by Granneman and Campbell, in vivo lipogenesis in BAT was also stimulated. Denervation decreased this response by 75%. As much as 91% of variations in IBAT weight may be accounted for by variations in body fat (Hervey and Tobin, 1983). Thus, BAT may serve as a lipid storage reservoir, removal of which necessitates increased storage in white adipose tissue.

In the present study, long-term feeding of a cafeteria diet was associated with substantial weight gain relative to Chow-fed rats and the activity of NPPase in IBAT was not elevated in dietary obese rats. Hill et al. (1983) were able to divide rats into two groups on the basis of their response to a high fat diet: one group showed increased efficiency of energy utilization, no change in diet-induced thermogenesis and positive weight gain; and a second group of animals of similar age, sex and strain showed

increased meal-associated thermogenesis but no change in efficiency of utilization and did not gain a significant amount of weight. Those authors found no correlation between diet-induced thermogenesis and oxygen consumption of IBAT.

Perhaps some of the disparity in the results and interpretations of the experiments discussed above can be explained with careful attention to time course, diet composition and individual or species variability. Rats fed a cafeteria diet for relatively long periods of time gain excess body weight in spite of increased thermogenesis in some, but not all, cases (Rothwell and Stock, 1979b).

Previous reports on the effects of fenfluramine on BAT activity and thermogenesis are conflicting. Lupien and Bray (1985) have reported increased GDP-binding in vivo with high dosages of d,l-fenfluramine (20 mg/kg), but not with lower dosages or with the high dosage in vitro. In those experiments, GDP-binding remained elevated with chronic (11 days) drug treatment and those authors claimed food intake had returned to normal within this time. We have observed however, that with dosages this high, complete anorectic tolerance usually does not develop, at least within 2 weeks. Furthermore, in the Lupien and Bray experiment, food intake of fenfluramine-treated animals appeared to be comparable to controls only on the last day (day 11); that is, intakes were significantly lower up to day 10.

Rothwell et al. (1982) found no effect of d,l-fenfluramine on NE uptake or $(\text{Na}^+-\text{K}^+)\text{ATPase}$ activity in BAT; however, basal ATPase appears to be regulated by alpha-1-noradrenergic receptors whereas the reported diet-induced increases require beta-noradrenergic receptor activation (Swann, 1984a). Beta-receptor activity may be sensitive to modification by corticosterone and gonadal steroid hormones as well as concentrations of NE and other factors that may be affected by fenfluramine and by diet. Furthermore, the obese state may be associated with changes in fluidity of a number of membranes including those of adipocytes (York et al., 1982) that may in turn reduce coupling of beta-receptors and adenylate cyclase and decrease stimulated $(\text{Na}^+-\text{K}^+)\text{ATPase}$ activity.

The activity of $(\text{Na}^+-\text{K}^+)\text{ATPase}$ in BAT may also be responsive to ovarian hormones (Bartness and Wade, 1984). Hormonal effects on ATPase have not been studied extensively. Adrenal hormones alter ATPase activity in some tissues (Charney and Donowitz, 1976) and may have an inductive effect, at least in developing animals (Huttenlocher and Amemiya, 1978). Estrogen stimulates (Krudsen, 1976) and progestins inhibit (LaBella et al., 1979; Yamamoto, 1978) ATPase activity in the anterior pituitary of ovariectomized rats. In vitro, insulin directly stimulates ATPase in some muscles under conditions of less than maximal activity (Gavryck et al., 1975; Clausen and Kohn, 1977).

Lipoprotein lipase (LPL) activity. The activity of retroperitoneal LPL was lower in dietary obese rats and higher in ovariectomized rats than in unoperated Chow-fed control rats. Four weeks of chronic d-fenfluramine administration significantly reduced retroperitoneal LPL activity in rats from each of the four dietary or surgical condition groups, including estradiol-treated rats. The reductions LPL activity were substantial, ranging from a 38% decrease in dietary obese rats to a 78% decrease in ovariectomized rats. At the end of the 2-week drug-free period, LPL activity in retroperitoneal adipose tissue of dietary obese and estradiol-treated rats that had received d-fenfluramine had returned to control levels. Lipoprotein lipase activity in retroperitoneal adipose tissue of ovariectomized and unoperated Chow-fed rats had been affected by d-fenfluramine treatment to a greater extent and had not returned to control levels within 2 weeks of the termination of drug treatment.

Lipoprotein lipase activity in inguinal WAT was also elevated in ovariectomized rats compared to rats in the other dietary or surgical condition groups; however, LPL activity in dietary obese rats was not significantly different from that of estradiol-treated rats or unoperated Chow-fed rats. Administration of d-fenfluramine for 4 weeks had no significant effect on inguinal LPL activity in any of the dietary or surgical condition groups.

Inguinal LPL activity was not closely correlated with inguinal adipocyte diameter in this study. Lipase activity was approximately 3-times greater in ovariectomized rats than in dietary obese or unoperated Chow-fed rats although inguinal adipocyte diameter was not significantly different among the three groups. Furthermore, inguinal adipocyte diameter was reduced in estradiol-treated rats compared to unoperated Chow-fed rats, but inguinal LPL activity was not significantly different among these groups. This is additional evidence for the suggestion that the relatively long-term obesity produced in this experiment resulted in adipocyte hyperplasia.

The activity of LPL in IBAT was higher in both ovariectomized and dietary obese rats and was lower in estradiol-treated rats compared to unoperated Chow-fed rats. Administration of d-fenfluramine for 4 weeks had no significant effect on LPL activity in IBAT and there were no significant differences in the activity of LPL in IBAT samples taken from previously d-fenfluramine-treated rats killed after a 2-week drug-free period compared IBAT samples taken at the termination of drug treatment or untreated controls. If increased LPL activity in IBAT of ovariectomized and dietary obese rats resulted in hyperplasia, this could partially explain the increased size of the IBAT pads in obese rats.

In the present study, LPL activity in the three types of adipose tissue examined was approximately 3-times higher in ovariectomized rats than in unoperated Chow-fed rats and administration of 2 ug estradiol benzoate/day reversed this effect of ovariectomy. This substantially elevated LPL activity in ovariectomized rats relative to other groups is consistent with previous reports of increased LPL activity in ovariectomized rats (Steingrimsdottir et al., 1980; Wade and Gray, 1978) and decreased activity with estradiol replacement has been reported also (Gray and Wade, 1981). Hamosh and Hamosh (1975) reported a 2-fold increase in LPL activity following ovariectomy in rats and this effect was reversed by physiological levels of estradiol.

The ovarian hormones have important effects on LPL activity that may mediate their effects on food intake and body weight (Wade and Gray, 1978). High affinity cytoplasmic binding sites have been found for [³H]estradiol (Wade and Gray, 1978) and a synthetic progestin ([³H]R5020) (Gray and Wade, 1979) in adipose tissue. Increases in LPL activity following ovariectomy precede changes in feeding behavior by about 12 hours (Steingrimsdottir et al., 1980). The increase in LPL activity following ovariectomy is prevented or reversed by administration physiological doses of estradiol (Gray and Wade, 1981). Gonadal steroids may also affect lipid metabolism by producing changes in hormone-sensitive lipase activity. Exogenously administered estrogen potentiates

catecholamine-stimulated lipolysis (Benoit et al., 1982; Hansen et al., 1980). Thus, the gonadal steroids may affect lipid storage in adipose tissue by producing reciprocal shifts in LPL and hormone-sensitive lipase activity.

The activity of LPL is affected by insulin levels; however, there is debate over the exact role and importance of insulin in regulation of LPL and over its relative importance in human obesity. Some studies suggest that plasma insulin levels are the major regulator of LPL activity (Cryer et al., 1976); however, other data suggest that the role of insulin on LPL activity is a permissive one (Eckel et al., 1978; Turkenkopf et al., 1982). In any event, insulin levels in vivo are well correlated with LPL activity and in vitro, LPL activity can be varied by changing insulin levels in the medium (Cryer et al., 1976). Furthermore, fat cell size is regulated by activity of LPL and hormone-sensitive lipase, and is an important determinant of insulin resistance which, in turn, affects insulin secretion.

The the effect of estrogen on LPL activity does not seem to be insulin-dependent, but may be a direct effect on protein synthesis (Wade and Gray, 1979). The results of the present experiment are consistent with an insulin-independent action of estrogen on LPL activity. Obese ovariectomized, ovariectomized-estradiol-treated and dietary obese rats were hyperinsulinemic; however, only ovariectomized rats had elevated LPL activity and in

retroperitoneal and brown adipose tissue, LPL activity of dietary obese rats was low compared to LPL activity in Chow-fed rats with normal insulin levels.

In the present study, LPL activity was decreased by approximately 50% in retroperitoneal white and interscapular brown, but not in inguinal, adipose tissues of dietary obese rats compared to unoperated Chow-fed rats. Previous reports on changes in LPL activity with diet-induced obesity are inconsistent: both increases and decreases have been reported. The differences in diet-induced changes in LPL activity in the different adipose tissues are consistent with previous reports of tissue differences in the responsiveness of LPL to changes in diet.

Lipoprotein lipase may be sensitive to changes in diet and body weight. Adipocyte LPL is related to cell size (Hietanen and Greenwood, 1977; Brunzell, 1979) and is elevated in several animal models of obesity as well as in a population of obese and formerly obese humans. The increase in LPL activity may occur in pre-obese animals prior to hyperphagia (Boulangue et al., 1979) and development of hyperinsulinemia (Turkenkopf et al., 1982). Obese humans have increased LPL activity per adipocyte but no difference in activity per gram tissue (Guy-Grand and Bigorie, 1975; Lithell and Boberg, 1978; Pykalisto et al., 1975; Taskinen and Nikkila, 1977). Whether or not this indicates a functional increase in activity is not clear.

The effects of d-fenfluramine on LPL activity have not been previously reported. In the present study, d-fenfluramine markedly decreased LPL activity in retroperitoneal adipose tissue of rats in each of the four dietary or surgical condition groups, but had no significant effect on LPL activity in inguinal or brown adipose tissues.

Carbohydrate and lipid metabolism. The fasted (12-18 hours) daytime plasma insulin concentrations of obese ovariectomized rats, estradiol-treated rats and dietary obese rats were elevated substantially compared to unoperated Chow-fed rats. Administration of d-fenfluramine for 4 weeks had no significant effect on plasma insulin concentrations of rats in the four dietary or surgical conditions and there were no significant differences in plasma insulin concentrations of d-fenfluramine-treated rats killed after a 2-week drug-free period compared to insulin concentrations of rats killed at the end of the treatment period or untreated control rats.

The marked hyperinsulinemia associated with ovariectomy or dietary obesity is consistent with previous reports (eg. Triscari et al., 1985). Hyperinsulinemia in estradiol-treated rats has also been reported: exogenously administered or endogenously secreted estradiol increase plasma insulin levels (Bailey and Matty, 1972; Matute and Kalkhoff, 1973). Increased insulin levels in obese ovariectomized rats and estradiol-treated rats may

reflect pancreatic synthesis and tissue sensitivity to insulin. Effects of estrogen on food intake and body weight are not critically dependent upon insulin.

Hyperinsulinemia is commonly found in a majority of obese animal models and in humans with longstanding obesity. The result of persistent hyperinsulinemia associated with obesity may be the development of insulin receptor insensitivity and Type II diabetes mellitus which constitutes a major health risk for the chronically obese.

Baseline insulin levels of rats made obese by feeding a high fat, high carbohydrate diet have been reported to be up to 6-times greater than in Chow-fed animals (Triscari et al., 1985). Hyperinsulinemia was not found in hyperphagic rats on similar diets that did not gain weight (Rothwell and Stock, 1981; Levin et al., 1983). Thus, increased body weight rather than caloric intake may be responsible for hyperinsulinemia or conversely, hyperinsulinemia may be necessary for excessive weight gain in dietary obese rats. There are species differences in insulin response to diet that may be related to differences in weight gain. Levin et al. (1983) have reported that male Sprague-Dawley rats did not gain weight and had normal insulin levels on the high fat, high carbohydrate diet; however, the high carbohydrate diet used in the present study caused female Sprague-Dawley rats to gain substantial amounts of weight and these rats also were hyperinsulinemic.

Chronic d-fenfluramine treatment had no significant effect on plasma insulin concentrations in any of the groups of rats in the present experiment. Previously reported effects of d,l-fenfluramine on insulin release are equivocal. In obese women treated chronically with 60 mg of fenfluramine given in three doses per day, a test dose of 40 mg suppressed insulin levels. Lowered insulin levels also have been reported in Type II diabetics (Asmal and Leary, 1975); however, a dose of 20 mg d,l-fenfluramine had no effect on insulin levels of normal weight, healthy subjects (Sulaiman and Johnson, 1973).

Dietary obese rats were hypoglycemic after being fasted overnight (12-18 hours) prior to the time they were killed. Four weeks of d-fenfluramine administration elevated fasting glucose concentrations of dietary obese rats to normal (125 mg/dl) levels, but had no significant effect on glucose concentrations of rats in the other dietary or surgical condition groups.

The hypoglycemia in dietary obese rats was not expected; however, the metabolic profile in these rats was marked by hyperinsulinemia and relatively high circulating corticosterone concentrations. The combination of hyperinsulinemia and hypercorticism is found in both humans and in some animal models of longstanding obesity.

Hyperinsulinemia has been associated with elevated plasma lipids. Elevated plasma FFA can inhibit insulin clearance (Smith,

1985) and also glucose uptake. With chronic d,l-fenfluramine treatment, FFA levels are correlated with drug levels. Thus elevated FFA may partially explain the increase in plasma glucose concentrations seen in dietary obese rats after 4 weeks of d-fenfluramine treatment. The d-fenfluramine associated increase in fasting glucose concentrations of dietary obese rats was sufficient to bring glucose concentrations within normal levels.

Previously reported effects of d,l-fenfluramine on carbohydrate metabolism in normal weight laboratory animals and humans include increased glucose uptake (Butterfield and Whichelow, 1968) and improved glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970). The insulin-dependent uptake of glucose in muscle is stimulated by d,l-fenfluramine and this appears to be mediated by 5-HT (Kirby and Turner, 1976; Turner, 1979; Turner et al., 1982). Racemic fenfluramine also has been reported to reduce fasting plasma glucose levels and improve glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970), effects that are not mediated by increased insulin release (Pasquine and Thenen, 1981).

Fasted plasma triglyceride concentrations were not significantly different among untreated control rats from the four dietary or surgical condition groups and there was no significant effect of the 4-week d-fenfluramine treatment regimen on plasma triglyceride concentrations. It has been reported that

d,l-fenfluramine is similarly ineffective on fasting triglyceride concentrations (Bizzi et al., 1973; Curtis-Prior, 1980, Garattini et al., 1975).

Rats with diet-induced obesity previously have been reported to have elevated FFA, glycerol and triglycerides. After 3 months on a high fat diet, FFA were increased by 38%; glycerol was increased by 41%; triglycerides were 80% above normal and ketogenesis was doubled (Triscari et al., 1985). It should be noted however, that the high fat diet used in that study would be more likely to result in higher plasma lipid concentrations than would the high carbohydrate diet used in the present study. As discussed above, elevations in plasma FFA may be related to hyperinsulinemia and hypoglycemia in dietary obese rats.

At high concentrations, d,l-fenfluramine causes an acute increase in plasma FFA and glycerol and a decrease in plasma triglycerides (Pawan, 1970) indicating that lipolysis is stimulated. This is also consistent with recent reports of prolonged stimulation of lipolysis by d-fenfluramine (Nicolaidis and Even, 1986).

Plasma corticosterone concentrations were elevated in untreated dietary obese rats, compared to the other dietary and surgical condition groups in this study. Chronic administration of d-fenfluramine substantially elevated plasma corticosterone concentrations of rats in all four dietary or surgical condition

groups compared to the corresponding group controls. By 2 weeks after the termination of d-fenfluramine treatment, plasma corticosterone concentrations had returned to control levels.

The glucocorticoids oppose the actions of insulin on gluconeogenesis, glycolysis, protein synthesis and glucose uptake in some tissues and decrease insulin sensitivity; however, they facilitate the actions of insulin in stimulating synthesis of glycogen and FFA (Amantruda et al., 1983) and increasing activity of LPL in adipose tissue (Robinson et al., 1985).

Exogenously administered glucocorticoids cause decreased appetite and weight loss reflected in both protein and lipid loss. When insulin is simultaneously administered, lipid storage and body weight are increased although protein wasting persists (Hausberger and Hausberger, 1958). This is similar to the clinical picture of Cushing's Syndrome in which protein wasting is associated with increased circulating glucose and high insulin levels that in turn promote excessive lipid storage. The consequences of this are truncal obesity, impaired glucose tolerance, diabetes and hypertension. In longstanding obesity, circulating corticosterone levels may be increased along with increased insulin levels, and this may be associated with the development of android obesity (Vague, 1983).

The glucocorticoids also affect insulin sensitivity. Injection of ACTH or glucocorticoids can produce a syndrome of insulin

resistant diabetes. Glucose intolerance is also observed in Cushing's syndrome and in patients undergoing glucocorticoid therapy (Munck, 1971; Pupo et al., 1966). This appears to be due to a decrease in receptor affinity for insulin and similarly, adrenalectomy causes an increase in insulin receptor affinity (Kahn et al., 1978).

Adrenocorticotrophic hormone (ACTH), which stimulates production and release of adrenal glucocorticoids; beta-lipotropin, the precursor of beta-endorphin, and melanotropin are produced from the same pro-hormone (pro-opiomelanocortin) and may be released simultaneously (Dubuc et al., 1975). Beta-endorphin has been shown to stimulate glucose-dependent insulin release (Ipp et al., 1978). Under conditions of prolonged stress, and perhaps as a natural consequence of aging, production and release of ACTH and beta-endorphin are increased. Thus, it has been suggested that this combination of glucocorticoid-stimulated gluconeogenesis and beta-endorphin enhancement of insulin release results in increased lipid storage relative to caloric intake (Margules, 1979).

Previous reports on the effects of d- and d,l-fenfluramine on plasma corticosterone have used high drug concentrations. In those studies, acute d,l-fenfluramine stimulated the release of adrenal glucocorticoids and catecholamines (Schettini et al., 1979). Brindley (1983) has found similar effects with d-fenfluramine. These effects appear to be mediated by increased

hypothalamic 5-HT availability which stimulates release of corticotropin releasing hormone (CRH). The time course of this action is parallel to that of anorexia and tolerance with daily injections of d,l-fenfluramine (Fuller et al, 1981). The physiological role of the 5-HT-stimulated CRH release is not clear; however, it appears to be involved in diurnal rhythmicity of adrenocortical activity (Fuller, 1981) which in turn, is correlated with feeding patterns in rats (Dallman, 1984). The role of this pathway in mediating acute stress responses is questionable: inhibition of 5-HT uptake does not decrease the rise in plasma corticosterone induced by insulin hypoglycemia or swim stress (Fuller and Snoddy, 1977).

The reported effects of chronic injections of high doses of d-fenfluramine on the adrenal hormones are quite different from the acute effects. Basal corticosterone levels are normal and the stress-evoked peaks in plasma corticosterone, catecholamines and FFA are substantially decreased (Brindley et al., 1985). Those effects were seen with with a high drug dosage (25 mg/kg/day), but not with a lower a dosage (2.5 mg/kg twice daily).

The elevated plasma corticosterone concentrations found under basal conditions after 4 weeks of chronic administration of 3 mg d-fenfluramine/kg/day via osmotic pump suggest that this method of administration produces a profile of chronic drug effects that are more like the acute effects seen with injection, particularly

where high doses are used. Other results of this study support this notion: brain 5-HT is not depleted and no apparent tolerance develops to weight loss with this treatment regimen. The clinical implications of this are important and the applicability of previous studies using intermittent administration of high doses of fenfluramine is challenged.

Experiment 2: Comparison of the Effects of Chronic d-Fenfluramine Treatment to Dietary Restriction

The effects of 4 weeks of administration of 3 mg d-fenfluramine/kg/day were compared to the effects of 4 weeks of dietary restriction in rats from the three dietary or surgical condition groups: OVX; DIO and CHOW. Experiment 2 was conducted simultaneously with Experiment 1 and the data on d-fenfluramine-treated and untreated control rats are the same as those presented in Experiment 1. The rats in the dietary restriction groups in each of the dietary or surgical conditions received no drug treatment, but were given access to only 75% of their baseline daily food intakes during the 4-week treatment period. These groups were included in order compare the effects of d-fenfluramine administration to dietary restriction in the absence of drug treatment.

Within both the DIO and CHOW conditions there were two groups of d-fenfluramine-treated rats and two groups of diet-restricted rats. At the end of the treatment period, one group of rats from the DIO and CHOW dietary restriction groups and one group of

d-fenfluramine-treated rats from those condition groups were killed. Tissue samples were collected for measurement of brain monoamines and for comparison of peripheral actions of d-fenfluramine to peripheral effects of dietary restriction and weight loss in the absence of drug treatment.

Rats in the untreated control groups and an additional group of diet-restricted or d-fenfluramine-treated rats from the DIO and CHOW conditions were kept alive for a 2-week drug-free posttreatment period during which rats from the restricted-diet groups were returned to an ad libitum feeding schedule. Measurements of food intake, body weight and food efficiency were continued. At the end of this 2-week period, these rats were killed and tissue samples were collected as before. The rats in the d-fenfluramine-treated and untreated control groups were the same as in Experiment 1. The purpose of this experiment was to compare the posttreatment effects of d-fenfluramine and dietary restriction in lean and obese rats.

Effects of d-Fenfluramine or Dietary Restriction on Food Intake,
Body Weight and Food Efficiency

Food intakes. Food intakes of d-fenfluramine-treated rats, converted to percentages of baseline total calories, were compared to food intakes of untreated controls and to the fixed (75% of baseline) intakes of diet-restricted rats in the three dietary or surgical condition groups. Mean 2-day caloric intakes on days 1-2, 7-8, 13-14 and 27-28 were analyzed by 3-way ANOVA with repeated measures design.

Food intakes on representative days throughout the treatment period, expressed as percentages of baseline, are shown for rats in the OVX (Figure 13), DIO (Figure 14) and CHOW (Figure 15) condition groups. On the first 2 days of administration, the suppression of food intake by d-fenfluramine was greater than 25%; but thereafter, food intakes of drug-treated rats in the OVX and CHOW groups returned to baseline levels ($F(2,12) = 30.44$, $p < .001$) whereas the intakes of drug-treated rats in the DIO group remained significantly below baseline through the second week of d-fenfluramine treatment ($F(3,18) = 36.20$, $p < .001$). The treatment by day of treatment interaction was significant ($F(6,36) = 18.66$, $p < .001$).

Food intakes during the 2-week posttreatment period were expressed as percentages of baseline intakes and analyzed by 3-way ANOVA with repeated measures design. Days 1-2, 7-8 and 13-14 of the posttreatment period were incorporated into the analysis comparing food intake of rats that had received d-fenfluramine or dietary restriction to that of untreated controls from the DIO (Figure 16) and CHOW (Figure 17) condition groups.

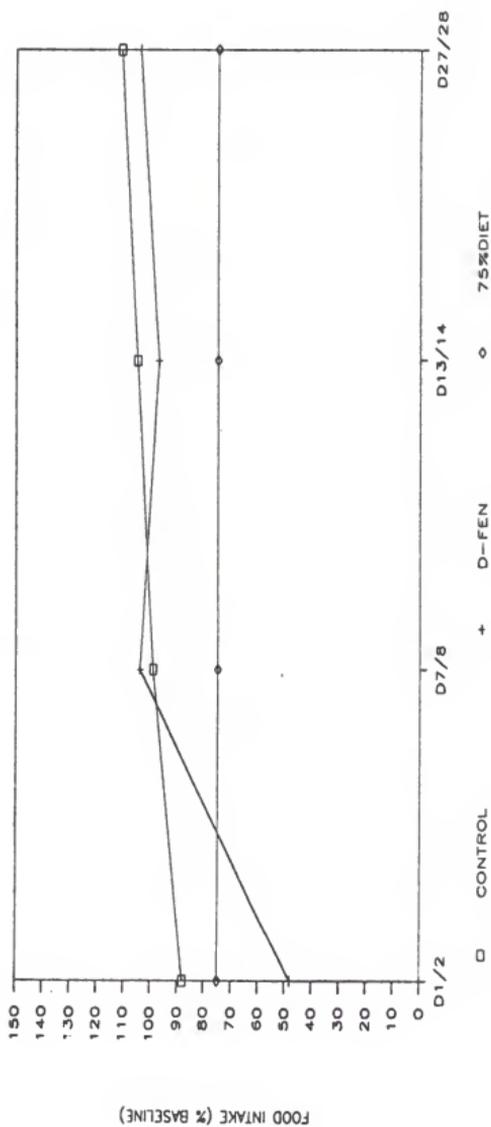


Figure 13. Effects of d-fenfluramine treatment on food intakes of ovariectomized rats (OVX) compared to a fixed (75% of baseline) restricted diet. Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN), diet-restricted rats (75% DIET) and untreated controls from the OVX condition group. Food intakes are expressed as percentages of baseline intakes on representative days during the 4-week treatment period (D1/2-D27/28).

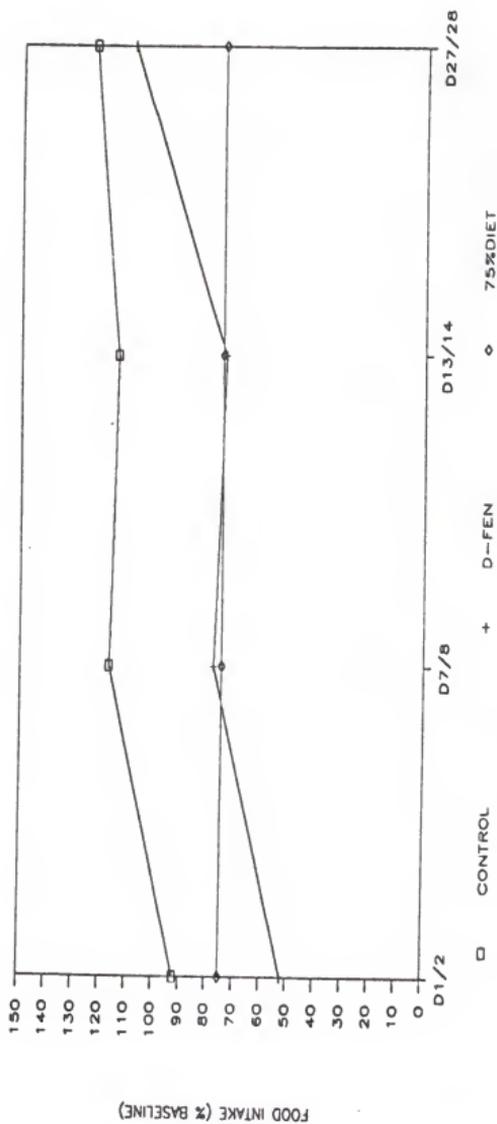


Figure 14. Effects of d-fenfluramine treatment on food intakes of dietary obese rats (DIO) compared to a fixed (75% of baseline) restricted diet. Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN), diet-restricted rats (75% DIER) and untreated controls from the DIO condition group. Food intakes are expressed as percentages of baseline intakes on representative days during the 4-week treatment period (D1/2-D27/28).

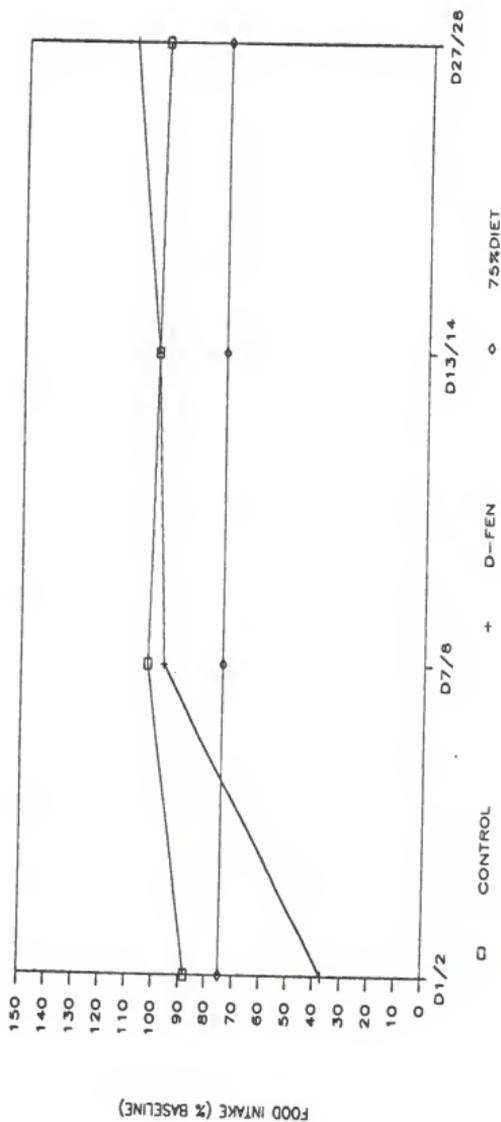


Figure 15. Effects of d-fenfluramine treatment on food intakes of unoperated Chow-fed rats (CHOW) compared to a fixed (75% of baseline) restricted diet. Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN), diet-restricted rats (75% DIET) and untreated controls from the CHOW condition group. Food intakes are expressed as percentages of baseline intakes on representative days during the 4-week treatment period (D1/2-D27/28).

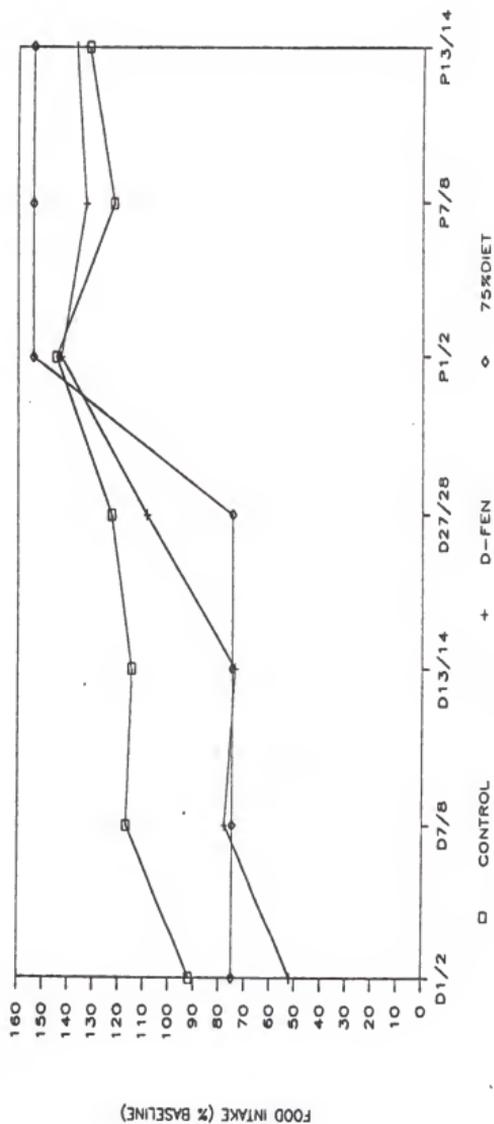


Figure 16. Effects of d-fenfluramine treatment on food intakes of dietary obese rats (DIO) compared to a fixed (75% of baseline) restricted diet. Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls from the DIO condition group. Food intakes are expressed as percentages of baseline intakes on representative days during the 4-week treatment period (days D1/2-D27/28) and the 2-week drug-free posttreatment period during which diet-restricted rats were returned to ad libitum feeding condition (days P1/2-P13/14).

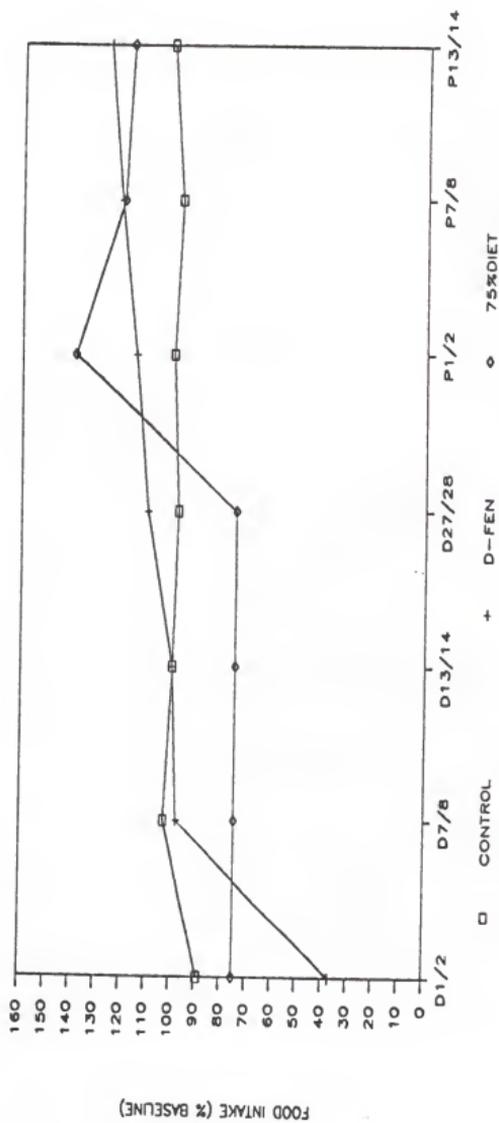


Figure 17. Effects of d-fenfluramine treatment on food intakes of unoperated Chow-fed rats (CHOW) compared to a fixed (75% of baseline) restricted diet. Shown are group mean 2-day food intakes of d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls from the CHOW condition group. Food intakes are expressed as percentages of baseline intake on representative days during the 4-week treatment period (days D1/2-D27/28) and the 2-week drug-free posttreatment period during which diet-restricted rats were returned to ad libitum feeding condition (days P1/2-P13/14).

During the posttreatment period, the food intakes of untreated control rats in the DIO group were approximately 33% above their baseline intakes whereas untreated control rats in the CHOW group maintained their intakes near baseline levels; therefore, there was a significant difference between the two conditions ($F(1,6) = 10.06, p = .02$). This difference between the conditions was also evident in the other treatment groups. Food intakes during the posttreatment period were not significantly affected by prior treatment with d-fenfluramine or dietary restriction ($F(2,12) = 1.80, p = .20$). The interaction between the treatment and dietary or surgical condition variables was not significant ($F(2,12) < 1.00, p > .25$). There were no significant changes in the food intakes over the 2-week posttreatment period ($F(2,12) = 1.45, p = .27$).

Body weights. Group mean weekly body weight changes and cumulative weight change are shown for rats in the OVX (Figure 18), DIO (Figure 19) and CHOW (Figure 20) condition groups. Untreated control rats in the DIO group gained more weight during the 4-week treatment period than control rats in the OVX and CHOW groups (2-way ANOVA, $F(2,91) = 30.07, p < .001$). Dietary restriction produced weight loss in the OVX and CHOW groups and significantly reduced weight gain in the DIO group compared to untreated

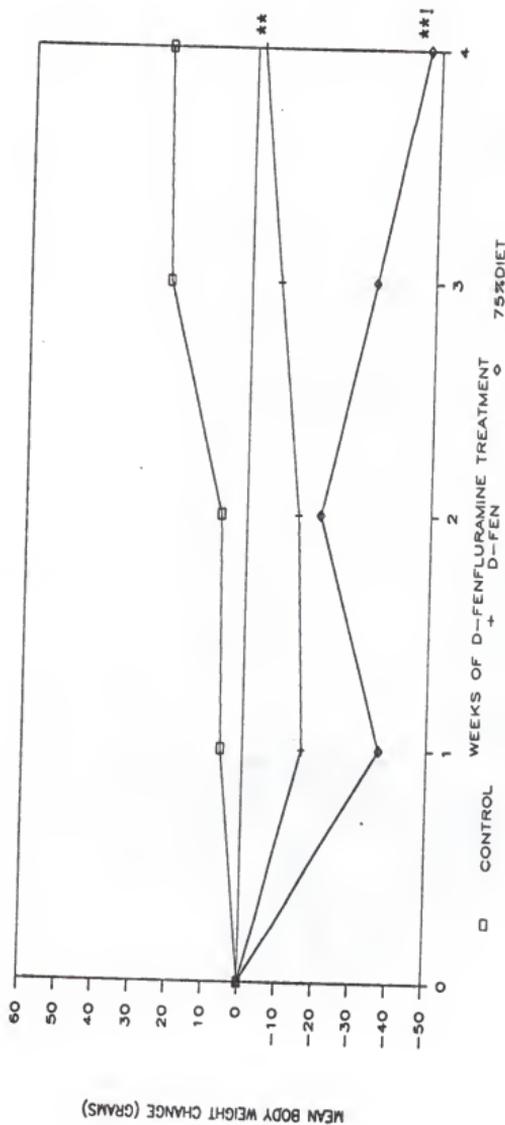


Figure 18. Effects of d-fenfluramine treatment or dietary restriction on body weights of ovariectomized (OVX) rats. Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls in the OVX condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4). ** Mean cumulative weight change during the 4-week treatment period different from untreated control group, $p < .01$, Newman-Kuel's post hoc t-test.
! Mean weight change during the 4-week treatment period different from D-FEN group, $p < .01$.

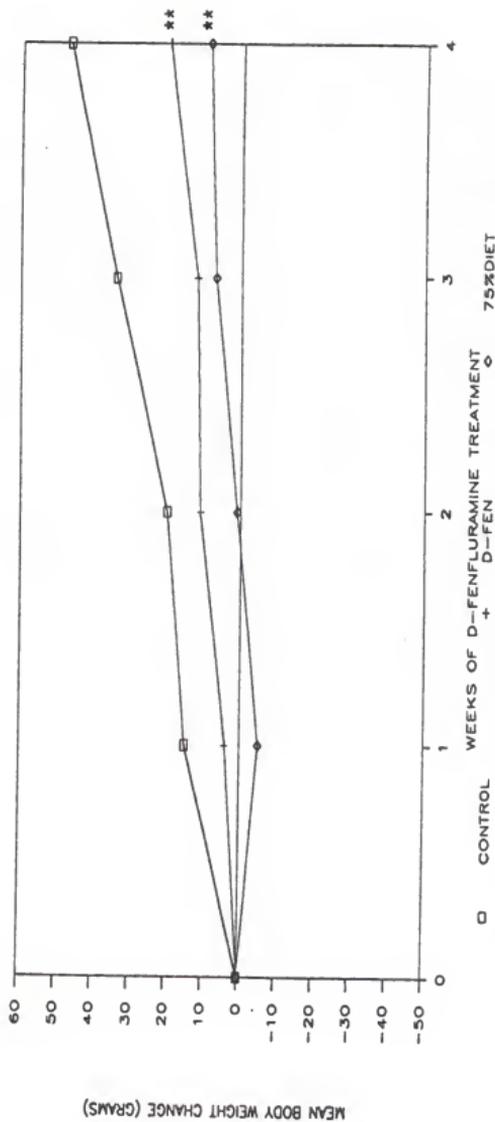


Figure 19. Effects of d-fenfluramine treatment or dietary restriction on body weights of dietary obese rats (DIO). Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls in the DIO condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4). ** Mean cumulative weight change during the 4-week treatment period different from untreated control group, $p < .01$, Newman-Kuel's post hoc t-test.

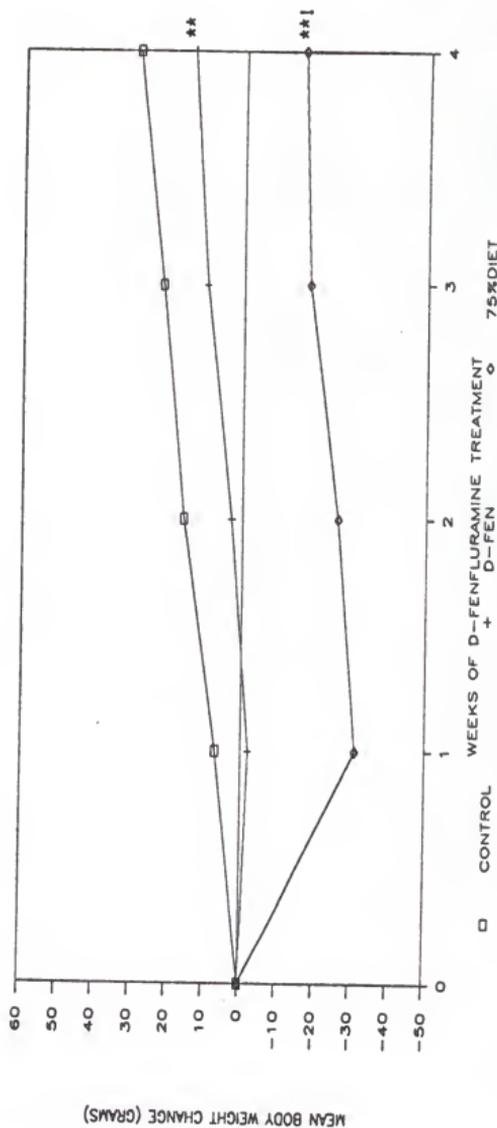


Figure 20. Effects of d-fenfluramine treatment or dietary restriction on body weights of unoperated Chow-fed rats (CHOW). Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls in the OVX condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4). ** Mean cumulative body weight change during the 4-week treatment period different from untreated control group, $p < .01$, Newman-Kuel's post hoc t-test.

! Mean cumulative body weight change during the 4-week treatment period different from D-FEN group, $p < .01$.

control rats in the corresponding groups. Fenfluramine treatment produced weight loss in the OVX group and significantly reduced weight gain in the DIO and CHOW groups. Dietary restriction was more effective than d-fenfluramine in reducing body weights of rats from the OVX and CHOW condition groups, but both treatments were similarly effective in rats from the DIO condition group ($F(2,91) = 48.06, p < .001$). There was a significant interaction between the dietary or surgical condition variable and the treatment variable ($F(4,91) = 3.00, p = .02$).

During the 2-week posttreatment interval, rats from both the DIO (Figure 21) and CHOW (Figure 22) condition groups that had received d-fenfluramine treatment or dietary restriction gained more weight than untreated controls in the corresponding condition groups ($F(2,38) = 8.98, p < .001$).

Food Efficiency. Rats in the OVX and CHOW groups that were on a restricted diet lost weight during the treatment period; therefore, it was not possible to estimate food efficiency from the ratio of [weight gain/kilocalorie ingested] for these rats during the treatment period.

During the posttreatment period, food efficiency was similar in rats that had received d-fenfluramine and in untreated control rats from both the DIO and CHOW condition groups ($F(1,38) = 2.18, p = .14$). The food efficiency of rats that had been on a

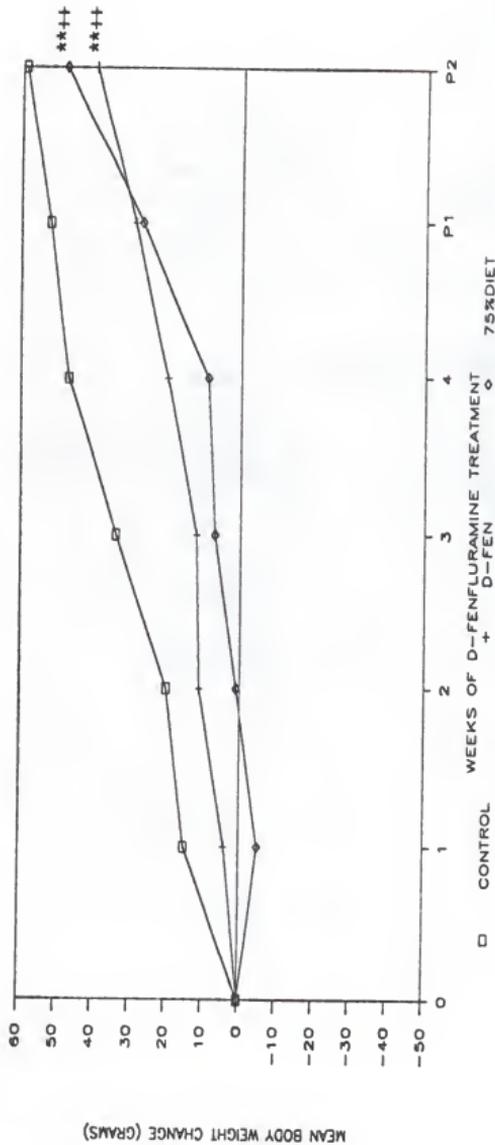


Figure 21. Treatment and posttreatment effects of d-fenfluramine or dietary restriction on body weights of dietary obese rats (DIO). Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls in the DIO condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4) and the 2-week drug-free posttreatment period (weeks P1-P2) during which diet-restricted rats were returned to ad libitum feeding conditions.

** Mean cumulative body weight change during the 4-week treatment period different from untreated control group, $p < .01$, Newman-Kuel's post hoc t-test.

++ Mean cumulative body weight change during the 2-week posttreatment period different from untreated control group, $p < .01$.

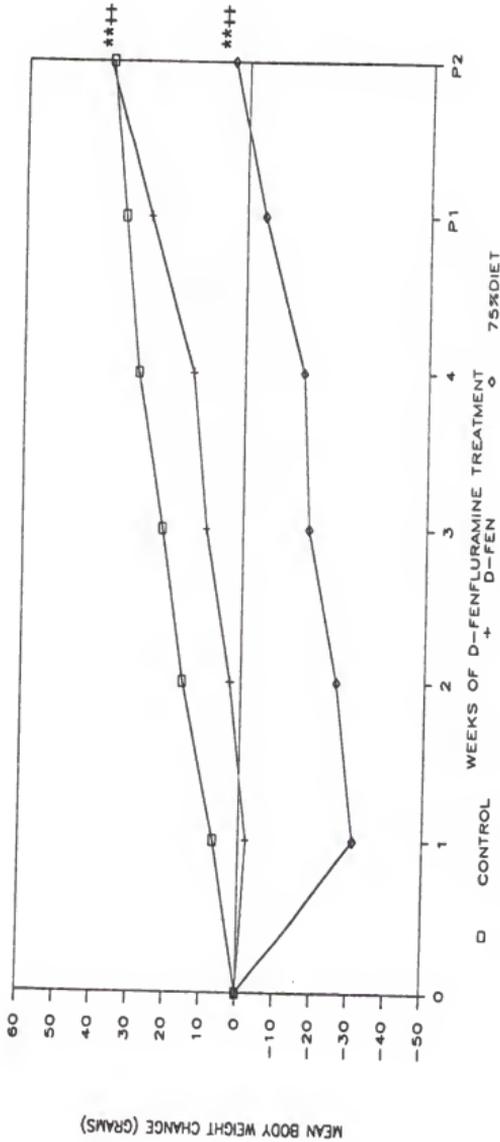


Figure 22. Treatment and posttreatment effects of d-fenfluramine treatment or dietary restriction on body weights of unoperated Chow-fed rats (CHOW). Shown are group mean weekly body weight changes (grams) for d-fenfluramine-treated rats (D-FEN), diet restricted rats (75% DIET) and untreated controls in the CHOW condition group. Mean weekly body weight changes are shown during the 4-week treatment period (weeks 1-4) and the 2-week drug-free posttreatment period (weeks P1-P2) during which diet-restricted rats were returned to ad libitum feeding conditions.

** Mean cumulative body weight change during the 4-week treatment period different from untreated control group, $p < .01$, Newman-Kuel's post hoc t-test.

++ Mean cumulative body weight change during the 2-week posttreatment period different from untreated control group, $p < .01$.

restricted diet was significantly elevated during this period in rats from both condition groups ($F(3,38) = 10.14$, $p < .001$) (Table 14).

Table 14. Treatment and Posttreatment Effects d-Fenfluramine or Dietary Restriction on Food Efficiency

Treatment	DIO	CHOW
Control	6.67 \pm 1.37	6.36 \pm 1.58
D-FEN(4wk)	7.73 \pm 1.41	7.23 \pm 1.49
D-FEN+2wk	7.79 \pm 2.39	7.44 \pm 1.97
75% Diet+2wk	14.94 \pm 1.81 **	12.32 \pm 3.00 **

Shown are group mean \pm S.E.M. food efficiency estimated from the ratio of [weight gained (grams)/kilocalorie food ingested] during the 4-week d-fenfluramine treatment period (D-FEN); during the 2-week drug-free posttreatment period for rats previously treated with d-fenfluramine (D-FEN + 2 wk) or dietary restriction (75% Diet + 2 wk) and untreated control values from the DIO and CHOW condition groups.

** Different from corresponding group, untreated control, Newman Kuel's post hoc t-test, $p < .01$.

Effects of d-Fenfluramine or Dietary Restriction on Adipocyte Size

The mean diameters of inguinal adipocytes from untreated control rats in the OVX, DIO and CHOW condition groups were not significantly different (2-way ANOVA, $F(2,26) = 2.00$, $p = .15$) (Table 15). Both dietary restriction and d-fenfluramine treatment reduced mean inguinal adipocyte diameter in all three condition groups ($F(2,26) = 68.00$, $p < .001$). The mean adipocyte diameter in

WAT from d-fenfluramine-treated rats in the DIO and CHOW groups was less than that of rats in those groups that had been diet-restricted, but the adipocyte diameter of diet-restricted rats in the OVX group was less than that of d-fenfluramine-treated rats in that group.

Table 15. Effects of d-Fenfluramine or Dietary Restriction on Inguinal Adipocyte Diameter

Treatment	OVX	DIO	CHOW
Control	.43 ± .020	.44 ± .010	.43 ± .010
D-FEN(4wk)	.34 ± .010**	.24 ± .010**	.23 ± .005**
75% Diet	.26 ± .003**!	.33 ± .005**!	.28 ± .001**!

Shown are group mean ± S.E.M. inguinal white adipocyte diameters (micrometers) at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after 4 weeks of dietary restriction (75% Diet) and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.

** Different from untreated controls in the corresponding condition group, $p < .01$, Newman Kuel's post hoc t-test.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

Two weeks after the return to ad libitum feeding the diameters of inguinal adipocytes of formerly diet-restricted rats in the DIO and CHOW condition groups had not returned to control levels (Table 16). The inguinal adipocyte diameters of d-fenfluramine-treated rats killed after a 2-week drug-free period had not returned to control levels, but were significantly greater than

adipocyte diameters of rats killed at the end of the treatment period ($F(4,34) = 50.00, p < .001$).

Table 16. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on Inguinal Adipocyte Diameter

Treatment	DIO	CHOW
Control	.44 ± .010	.43 ± .010
D-FEN(4wk)	.24 ± .010**	.23 ± .005**
75% Diet	.33 ± .005**!	.28 ± .001**!
D-FEN+2wk	.34 ± .010**!	.39 ± .010**!
75% Diet+2wk	.31 ± .020**	.31 ± .005**!!

Shown are group mean ± S.E.M. inguinal white adipocyte diameters (micrometers) at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or 4 weeks of dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values for rats from the DIO and CHOW dietary or surgical condition groups.

** Different from untreated controls in the corresponding condition group, $p < .01$, Newman Kuel's post hoc t-test.

! Different from corresponding D-FEN (4 wk) group.

!! Different from corresponding 75% Diet group, $p < .01$.

The diameters of brown adipocytes (Table 17) were not significantly different among the OVX, DIO and CHOW dietary or surgical condition groups (2-way ANOVA, $F(2,26) < 1.00$, $p > .25$) and were not significantly affected by d-fenfluramine treatment or by dietary restriction ($F(2,26) = < 1.00$, $p > .25$).

Table 17. Effects of d-Fenfluramine or Dietary Restriction on Interscapular Brown Adipocyte Diameter

Treatment	OVX	DIO	CHOW
Control	.08 ± .020	.06 ± .015	.04 ± .010
D-FEN(4wk)	.05 ± .010	.05 ± .005	.05 ± .020
75% Diet	.05 ± .005	.05 ± .002	.05 ± .005

Shown are group mean ± S.E.M. interscapular brown adipocyte diameters (micrometers) at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet) and untreated control values from rats in the OVX, DIO and CHOW dietary or surgical condition groups.

There were no significant differences in cell diameters of interscapular brown adipocytes from rats in the DIO and CHOW condition groups at the end of the 2-week posttreatment period ($F(4,28) < 1.00$, $p > .25$) (Table 18).

Table 18. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on Interscapular Brown Adipocyte Diameter

Treatment	DIO	CHOW
Control	.06 ± .015	.04 ± .010
D-FEN(4wk)	.05 ± .005	.05 ± .020
75% Diet	.05 ± .002	.05 ± .005
D-FEN+2wk	.05 ± .006	.05 ± .005
75% Diet+2wk	.05 ± .010	.05 ± .007

Shown are group mean ± S.E.M. interscapular brown adipocyte diameters (micrometers) at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values in rats from the DIO and CHOW condition groups.

Effects of d-Fenfluramine or Dietary Restriction on Brain 5-HT and DA

The effects of d-fenfluramine treatment on concentrations of 5-HT, 5-HIAA, DA and DOPAC in rat telencephalon and hypothalamus were compared to dietary restriction and untreated control values by HPLC-ED. Regional concentrations of each monoamine and the ratios of metabolite/neurotransmitter were compared among d-fenfluramine-treated or diet-restricted rats and untreated controls by 1-way ANOVA.

Brain 5-HT concentrations are shown in Table 19. Neither continuous delivery of d-fenfluramine by osmotic minipump nor dietary restriction for 4 weeks had a significant effect on the concentration of 5-HT in the telencephalon ($F(2,63) < 1.00$, $p > .25$). Dietary restriction produced a slight, but statistically significant decrease in the concentration of 5-HIAA ($F(2,56) = 5.06$, $p = .009$) and in the ratio of 5-HIAA/5-HT ($F(2,56) = 6.84$, $p = .002$) in the telencephalon. In the hypothalamus, d-fenfluramine treatment, but not dietary restriction, produced a significant increase in the concentrations of 5-HT ($F(2,74) = 3.62$, $p = .03$), but the effect on 5-HIAA was not statistically significant in this experiment ($F(2,70) = 2.65$, $p = .08$). The ratio of 5-HIAA/5-HT in the hypothalamus was not significantly affected by either treatment ($F(2,70) < 1.00$, $p > .25$).

The concentrations of brain 5-HT in rats that received d-fenfluramine treatment followed by a 2-week drug-free period or dietary restriction followed by a 2-week ad libitum feeding period were compared to those of rats killed at the end of the treatment period and to untreated controls by 1-way ANOVA (Table 20). In the telencephalon, there were no significant differences among the treatment groups in the concentrations of 5-HT ($F(4,96) > 1.00$, $p > .25$), but the concentration 5-HIAA was lower in rats killed at the end of 4 weeks of dietary restriction than in the other treatment groups ($F(4,89) = 3.12$, $p = .02$). The ratio of 5-HIAA/5-HT

Table 19. Effects of d-Fenfluramine or Dietary Restriction on Brain Serotonin (5-HT) and 5-HIAA Concentrations

<u>Telencephalon</u>			
Treatment	5-HT	5-HIAA	5-HIAA/5-HT
Control	7.39 ± 0.38	1.49 ± 0.11	20 ± 1%
D-FEN(4wk)	6.98 ± 0.41	1.13 ± 0.13	16 ± 2%
75% Diet	7.99 ± 0.70	0.84 ± 0.05 *	12 ± 2% *
<u>Hypothalamus</u>			
Treatment	5-HT	5-HIAA	5-HIAA/5-HT
Control	7.51 ± 0.46	2.51 ± 0.20	34 ± 2%
D-FEN(4wk)	9.48 ± 0.53 *	3.37 ± 0.32 *	36 ± 3%
75% Diet	8.23 ± 0.56	2.70 ± 0.29	34 ± 3%

Shown are group mean ± S.E.M. concentrations (nanomoles/gram wet tissue) of 5-HT and 5-HIAA in the telencephalon and hypothalamus at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after 4 weeks of dietary restriction (75% Diet) and untreated control values. Also shown are the ratios of 5-HIAA/5-HT expressed as percentages ± S.E.M.

* Different from untreated control values, $p < .05$, Newman Kuel's post hoc t-test, $p < .05$.

Table 20. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on Brain Serotonin (5-HT) and 5-HIAA Concentrations

<u>Telencephalon</u>			
Treatment	5-HT	5-HIAA	5-HIAA/5-HT
Control	7.39 ± 0.38	1.49 ± 0.11	$20 \pm 1\%$
D-FEN(4wk)	6.98 ± 0.41	1.13 ± 0.13	$16 \pm 2\%$
75% Diet	7.99 ± 0.70	$0.84 \pm 0.05 *$	$12 \pm 2\% *$
D-FEN+2wk	7.56 ± 0.73	1.24 ± 0.09	$18 \pm 2\%$
75% Diet+2wk	8.26 ± 0.69	1.11 ± 0.13	$13 \pm 1\% *$
<u>Hypothalamus</u>			
Treatment	5-HT	5-HIAA	5-HIAA/5-HT
Control	7.51 ± 0.46	2.51 ± 0.20	$34 \pm 2\%$
D-FEN(4wk)	9.48 ± 0.53	3.37 ± 0.32	$36 \pm 3\%$
75% Diet	8.23 ± 0.56	2.70 ± 0.29	$34 \pm 34\%$
D-FEN+2wk	7.49 ± 0.52	2.90 ± 0.21	$39 \pm 2\%$
75% Diet+2wk	7.67 ± 0.81	2.92 ± 0.38	$42 \pm 5\%$

Shown are group mean \pm S.E.M. concentrations (nanomoles/gram wet tissue) of 5-HT and 5-HIAA in the telencephalon and hypothalamus at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values. Also shown are the ratios of 5-HIAA/5-HT expressed as percentages \pm S.E.M.

* Different from untreated control values, $p < .05$, Newman Kuel's post hoc t-test.

in the telencephalon was significantly lower in rats that were diet-restricted and remained lower after 2-weeks of ad libitum feeding ($F(4,89) = 5.67, p < .001$).

Two weeks after the termination of d-fenfluramine treatment, the concentration of 5-HT in the hypothalamus had returned to control levels and the differences among the five treatment groups were not statistically significant ($F(4,108) = 2.21, p = .07$). There were no significant differences in the hypothalamic concentrations of 5-HIAA among the treatment groups ($F(4,104) = 1.25, p > .25$) or in the ratio of 5-HIAA/5-HT ($F(4,104) = 1.26, p > .25$).

Brain DA concentrations of d-fenfluramine-treated rats, diet-restricted rats and untreated controls from the OVX, DIO and CHOW surgical or dietary condition groups are shown in Table 21. There were no significant effects of 4 weeks of dietary restriction or d-fenfluramine treatment on the concentration of DA in the telencephalon (1-way ANOVA, ($F(2,39) < 1.00, p > .25$) or hypothalamus ($F(2,46) = 1.10, p > .25$); on the concentration of DOPAC in the telencephalon ($F(2,30) = 1.26, P > .25$) or hypothalamus ($F(2,43) < 1.00, p > .25$) or in the ratio of DOPAC/DA in the telencephalon ($F(2,30) < 1.00, p > .25$) or hypothalamus ($F(2,40) < 1.00, p > .25$).

Shown in Table 22, are the concentrations of brain DA in rats that received d-fenfluramine treatment followed by a 2-week drug-free period or dietary restriction followed by a 2-week

Table 21. Effects of d-Fenfluramine or Dietary Restriction on Brain Dopamine (DA) and DOPAC Concentrations

<u>Telencephalon</u>			
Treatment	DA	DOPAC	DOPAC/DA
Control	4.22 ± 0.51	1.10 ± 0.20	$27 \pm 4\%$
D-FEN(4wk)	3.99 ± 0.44	0.87 ± 0.13	$25 \pm 5\%$
75% Diet	3.46 ± 0.55	0.88 ± 0.11	$22 \pm 4\%$
<u>Hypothalamus</u>			
Treatment	DA	DOPAC	DOPAC/DA
Control	1.51 ± 0.17	0.77 ± 0.08	$60 \pm 4\%$
D-FEN(4wk)	1.42 ± 0.10	1.12 ± 0.29	$68 \pm 6\%$
75% Diet	2.03 ± 0.17	0.79 ± 0.15	$39 \pm 18\%$

Shown are group mean concentrations (nanomoles/gram wet tissue) of DA and DOPAC in the telencephalon and hypothalamus at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet) and untreated control values. Also shown are the ratios of DOPAC/DA expressed as percentages \pm S.E.M.

Table 22. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on Brain Dopamine (DA) and DOPAC Concentrations

<u>Telencephalon</u>			
Treatment	DA	DOPAC	DOPAC/DA
Control	4.22 ± 0.51	1.10 ± 0.20	27 ± 4%
D-FEN(4wk)	3.99 ± 0.44	0.87 ± 0.13	25 ± 5%
75% Diet	3.46 ± 0.55	0.88 ± 0.11	22 ± 4%
D-FEN+2wk	3.94 ± 0.54	1.02 ± 0.31	23 ± 5%
75% Diet+2wk	4.53 ± 0.51	1.21 ± 0.13	20 ± 3%
<u>Hypothalamus</u>			
Treatment	DA	DOPAC	DOPAC/DA
Control	1.51 ± 0.17	0.77 ± 0.08	60 ± 4%
D-FEN(4wk)	1.42 ± 0.10	1.12 ± 0.29	68 ± 6%
75% Diet	2.03 ± 0.17	0.79 ± 0.15	39 ± 18%
D-FEN+2wk	1.26 ± 0.20	0.94 ± 0.09	52 ± 5%
75% Diet+2wk	1.50 ± 0.22	0.73 ± 0.16	59 ± 6%

Shown are group mean concentrations (nanomoles/gram wet tissue) of DA and DOPAC in the telencephalon and hypothalamus at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values. Also shown are the ratios of DOPAC/DA expressed as percentages ± S.E.M.

ad libitum feeding period compared to those of rats killed at the end of the treatment period and to untreated controls. Results were compared by 1-way ANOVA. In the telencephalon, there were no significant differences among the treatment groups in the concentrations of DA ($F(4,58) >1.00$, $p >.25$); DOPAC ($F(4,61) = 1.18$, $p >.25$) or in the ratio of DOPAC/DA ($F(4,46) = 1.05$, $p >.25$). In the hypothalamus, there were no significant differences among the treatment groups in the concentrations of DA ($F(4,57) = 2.43$, $p = .06$); DOPAC ($F(4,61) <1.00$, $p >.25$) or in the ratio of DOPAC/DA ($F(4,55) <1.00$, $p >.25$).

Comparison of the Peripheral Actions of d-Fenfluramine to the Effects of Dietary Restriction

The peripheral effects of 4 weeks of d-fenfluramine administration via osmotic minipump were compared to the peripheral effects of 4 weeks on a restricted diet that allowed rats access to 75% of their baseline caloric intakes and these two treatments were compared to untreated control values for the OVX, DIO and CHOW dietary or surgical condition groups. These peripheral effects were measured in rats from the OVX, DIO and CHOW condition groups killed at the end of the 4-week drug or restricted diet treatment. Additional groups of rats in the DIO and CHOW conditions were killed after a 2-week drug-free, ad libitum feeding period.

NPPase activity. The activity of NPPase (nanomoles/minute/milligram protein) was measured in interscapular brown adipose

tissue (IBAT) (Table 23) and gastrocnemius muscle of d-fenfluramine-treated rats, diet-restricted rats and untreated control rats from the OVX, DIO and CHOW dietary or surgical conditions.

Table 23. Effects of d-Fenfluramine or Dietary Restriction on NPPase Activity in Brown Adipose Tissue

Treatment	OVX	DIO	CHOW
Control	1.44 ± 0.13 ⁺⁺	5.87 ± 0.64	6.00 ± 1.10
D-FEN(4wk)	5.82 ± 0.43 ^{**}	6.42 ± 0.56	10.73 ± 1.42 [*]
75% Diet	1.72 ± 0.77 [!]	5.23 ± 0.98	5.04 ± 0.60 [!]

Shown are group mean ± S.E.M. NPPase activity in brown adipose tissue expressed as nanomoles/minute/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet) and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.

* Different from untreated control rats in the corresponding condition group, $p < .05$, Newman Kucl's post hoc t-test.

** Different from untreated control rats in the corresponding condition group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

++ Different from CHOW untreated control group, $p < .01$.

The activity of NPPase in IBAT was lower in untreated control rats from the OVX group compared to control rats in the DIO and CHOW condition groups (2-way ANOVA, $F(2,49) = 40.25$, $p < .001$). Dietary restriction had no significant effect on NPPase activity in IBAT; however, administration of d-fenfluramine for 4 weeks significantly increased NPPase activity in IBAT of rats from the OVX and CHOW groups, but did not affect the activity of this

enzyme significantly in rats from the DIO condition group ($F(2,45) = 6.33, p = .004$). There was a significant interaction between the dietary or surgical condition variable and the treatment variable ($F(4,45) = 5.24, p = .003$).

Table 24. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on NPPase Activity in Brown Adipose Tissue

Treatment	DIO	CHOW
Control	5.87 ± 0.64	6.00 ± 1.10
D-FEN(4wk)	6.42 ± 0.56	10.73 ± 1.42*
75% Diet	5.23 ± 0.98	5.04 ± 0.60!
D-FEN+2wk	5.03 ± 0.83	4.19 ± 0.82!
75% Diet+2wk	5.20 ± 1.13	5.14 ± 0.86

Shown are group mean ± S.E.M. NPPase activity in brown adipose tissue expressed as nanomoles/minute/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values for rats from the DIO and CHOW condition groups.

* Different from untreated control rats in the corresponding condition group, $p < .05$, Newman-Kuel's post hoc t-test.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

By the end of the 2-week drug-free period, NPPase activity in IBAT of rats in the CHOW condition group that had been treated with d-fenfluramine had returned to control levels and was

significantly lower than NPPase activity at the end of the treatment period ($F(4,51) = 4.64, p = .003$) (Table 24). There were no significant effects of dietary restriction on NPPase activity in IBAT of rats killed at the end of the 4-week treatment period or of rats killed after a 2-week ad libitum feeding posttreatment period. The interaction between the treatment and dietary or surgical condition group variables was not significant ($F(4,51) = 1.98, p = .11$).

The activity of NPPase in gastrocnemius muscle (Table 25) was significantly decreased in untreated control rats from the OVX and DIO groups compared to enzyme activity in control rats from the CHOW group (2-way ANOVA, $F(2,49) = 40.25, p < .001$). Administration of d-fenfluramine for 4 weeks significantly increased the activity of muscle NPPase in rats from the OVX group ($F(2,49) = 37.07, p < .001$), but the increase was not sufficient to raise enzyme activity in these rats to the same level as in untreated control rats from the CHOW group. Administration of d-fenfluramine had no significant effect on muscle NPPase activity in rats from the DIO and CHOW dietary or surgical condition groups compared to untreated controls in those groups, thus enzyme activity in d-fenfluramine-treated rats from the DIO group remained below the level of activity in untreated control rats from the CHOW group. Four weeks of dietary restriction produced a significant decrease in muscle NPPase activity of rats in the DIO and CHOW groups, but had

no effect on NPPase activity of rats in the OVX condition groups ($F(2,49) = 18.09$, $p < .001$). There was a significant interaction between the dietary or surgical condition variable and the treatment variable ($F(4,49) = 15.52$, $p < .001$).

Table 25. Effects of d-Fenfluramine or Dietary Restriction on NPPase Activity in Gastrocnemius Muscle

Treatment	OVX	DIO	CHOW
Control	$1.06 \pm 0.12^{++}$	$2.62 \pm 0.51^{++}$	4.75 ± 0.62
D-FEN(4wk)	$2.00 \pm 0.21^{**}$	2.24 ± 0.21	5.62 ± 0.62
75% Diet	$0.85 \pm 0.15!$	$1.33 \pm 0.07^{**}!$	$1.72 \pm 0.21^{**}!$

Shown are group mean \pm S.E.M. NPPase activity in gastrocnemius muscle expressed as nanomoles/minute/milligram protein at the end of 4 weeks of d-fenfluramine (D-FEN) treatment or 4 weeks of dietary restriction (75% Diet) and untreated control values in rats from the OVX, DIO and CHOW dietary or surgical condition groups.

** Different from untreated control rats in the corresponding condition group, $p < .01$, Newman Kuel's post hoc t-test.

++ Different from CHOW untreated control group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

Muscle NPPase activity was lower in untreated control rats from the DIO condition group than untreated control rats in the CHOW condition group ($F(1,55) = 22.69$, $p < .001$). Dietary restriction produced a decrease in the activity of muscle NPPase in both the DIO and CHOW condition groups that was reversed by a 2-week period of ad libitum feeding in rats from the CHOW condition group, but not in rats from the DIO group ($F(4,55) = 13.95$,

p <.001) (Table 26). There was no significant effect of d-fenfluramine treatment on muscle NNPase activity of rats in the DIO and CHOW condition groups that were killed at the end of drug treatment or after a 2-week drug-free period.

Table 26. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on NNPase Activity in Gastrocnemius Muscle

Treatment	DIO	CHOW
Control	2.62 ± 0.51++	4.75 ± 0.62
D-FEN(4wk)	2.24 ± 0.21	5.62 ± 0.62
75% Diet	1.33 ± 0.07**!	1.72 ± 0.21**!
D-FEN+2wk	2.38 ± 0.17	5.12 ± 1.00
75% Diet+2wk	1.58 ± 0.21**	3.80 ± 0.37!!

Shown are group mean ± S.E.M. NNPase activity in gastrocnemius muscle expressed as nanomoles/minute/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values from rats in the DIO and CHOW dietary or surgical condition groups.

** Different from untreated control rats in the corresponding condition group, p <.01, Newman Kuel's post hoc t-test.

++ Different from CHOW untreated control group, p <.01.

! Different from corresponding D-FEN (4 wk) group, p <.01

!! Different from corresponding 75% Diet group, p <.01.

Lipoprotein lipase (LPL) activity. The activity of lipoprotein lipase (LPL) was measured in retroperitoneal and inguinal white adipose tissue (WAT) and in interscapular brown adipose tissue (IBAT) in d-fenfluramine-treated rats and diet-restricted rats from the OVX, DIO and CHOW condition groups. Lipase activity was expressed as micromoles FFA/hour/milligram protein in retroperitoneal and inguinal WAT and in interscapular brown adipose tissue.

The activity of retroperitoneal LPL (Table 27) was significantly lower in untreated control rats from the DIO group and higher in control rats from the OVX group compared to control rats in the CHOW group (2-way ANOVA $F(2,42) = 2.72, p = .04$). After 4 weeks of d-fenfluramine administration, LPL activity was decreased significantly in all three dietary and surgical condition groups ($F(2,42) = 4.04, p = .02$). There were no significant effects of dietary restriction on retroperitoneal LPL activity. There was not a significant interaction between the dietary or surgical condition variable and the treatment variable ($F(4,42) < 1.00, p > .25$).

Retroperitoneal LPL activity in the d-fenfluramine-treated and diet-restricted rats in the DIO and CHOW condition groups that were killed at the end of the treatment period or after a two-week drug-free, ad libitum feeding period and untreated control rats in those condition groups is shown in Table 28.

Table 27. Effects of d-Fenfluramine or Dietary Restriction on LPL Activity in Retroperitoneal White Adipose Tissue

Treatment	OVX	DIO	CHOW
Control	2.27 ± .62++	0.36 ± .03++	0.87 ± .21
D-FEN(4wk)	0.50 ± .08**	0.26 ± .04**	0.29 ± .09**
75% Diet	1.59 ± .13!	0.35 ± .03!	0.64 ± .08!

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity in retroperitoneal adipose tissue expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet) and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.

** Different from untreated control rats in the corresponding dietary or surgical condition group, $p < .01$, Newman-Kuel's post hoc t-test.

! Different from corresponding D-FEN group, $p < .01$.

++ Different from CHOW untreated control group, $p < .01$.

The activity of retroperitoneal LPL was significantly lower in untreated control rats from the DIO group compared to control rats in the CHOW group (2-way ANOVA $F(1,46) = 3.66$, $p = .05$). After 4 weeks of d-fenfluramine administration, LPL activity was decreased significantly in both the DIO and CHOW condition groups ($F(4,46) = 3.99$, $p = .01$). By 2 weeks after the termination of d-fenfluramine treatment, retroperitoneal LPL activity in rats from the DIO condition groups was elevated significantly above enzyme activity at the end of the treatment period and was not significantly different from control levels. Lipase activity in

d-fenfluramine-treated rats from the CHOW condition group had recovered significantly by the end of the 2-week drug-free period, but remained significantly lower than control enzyme activity. There was not a significant interaction between the dietary or surgical condition variable and the treatment variable ($F(4,46) = 1.34, p > .25$).

Table 28. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on LPL Activity in Retroperitoneal White Adipose Tissue

Treatment	DIO	CHOW
Control	0.36 ± .03++	0.87 ± .21
D-FEN(4wk)	0.26 ± .04**	0.29 ± .09**
75% Diet	0.35 ± .03	0.64 ± .08
D-FEN+2wk	0.54 ± .21!	0.49 ± .14*
75% Diet +2wk	0.50 ± .09	0.84 ± .21

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity in retroperitoneal adipose tissue expressed as micromoles FFA/hour/milligram protein in rats from the DIO and CHOW condition groups that were killed at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values.

* Different from untreated control rats in the corresponding condition group, $p < .05$, Newman-Kuel's post hoc t-test.

** Different from untreated control rats in the corresponding condition group, $p < .01$.

! Different from corresponding D-FEN (4 wk) group, $p < .01$.

++ Different from untreated CHOW group control, $p < .01$.

Lipoprotein lipase activity in inguinal WAT (Table 29) was significantly elevated in untreated control rats from the OVX group compared to control rats in the DIO and CHOW dietary or surgical condition groups ($F(2,37) = 20.88, p < .001$). Neither administration of d-fenfluramine nor dietary restriction imposed for 4 weeks had a significant effect on inguinal LPL activity in any of the three dietary or surgical condition groups ($F(2,37) < 1.00, p > .25$).

Table 29. Effects of d-Fenfluramine or Dietary Restriction on LPL Activity in Inguinal White Adipose Tissue

Treatment	OVX	DIO	CHOW
Control	1.21 ± .19++	0.32 ± .05	0.39 ± .05
D-FEN(4wk)	1.20 ± .23	0.38 ± .01	0.38 ± .12
75% Diet	1.52 ± .14	0.65 ± .22	0.52 ± .05

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity in inguinal adipose tissue expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet) and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.

++ Different from CHOW untreated control group, $p < .01$, Newman Kuel's post hoc t-test.

In the comparison of inguinal LPL activities of rats in the DIO and CHOW groups that were killed at the end of 4 weeks of d-fenfluramine treatment or dietary restriction; after a 2-week

drug-free, ad libitum feeding period and untreated control values (Table 30), there were no significant differences between the two condition groups ($F(1,46) = 3.200, p = .08$) and no significant treatment effects ($F(4,46) = 2.68, p = .11$).

Table 30. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on LPL Activity in Inguinal White Adipose Tissue

Treatment	DIO	CHOW
Control	0.32 ± .05	0.39 ± .05
D-FEN(4wk)	0.38 ± .01	0.38 ± .12
75% Diet	0.65 ± .22	0.52 ± .05
D-FEN+2 wk	0.48 ± .01	0.38 ± .06
75% Diet+2wk	0.32 ± .05	0.39 ± .05

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity in inguinal adipose tissue expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values for rats from the DIO and CHOW condition groups.

The activity of LPL in IBAT (Table 31) was significantly higher in untreated control rats from the OVX and DIO groups compared to control rats in the CHOW group ($F(2,43) = 14.00, p < .001$). Neither administration of d-fenfluramine nor dietary

restriction for 4 weeks had a significant effect on the activity of LPL in IBAT ($F(2,43) < 1.00, p > .25$).

Table 31. Effects of d-Fenfluramine or Dietary Restriction on LPL Activity in Interscapular Brown Adipose Tissue

Treatment	OVX	DIO	CHOW
Control	.28 ± .03++	.17 ± .04+	.10 ± .01
D-FEN(4wk)	.22 ± .03	.09 ± .01	.12 ± .02
75% Diet	.39 ± .04	.09 ± .02	.09 ± .01

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity in brown adipose tissue expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or after 4 weeks of dietary restriction (75% Diet) and untreated control values from rats in the OVX, DIO and CHOW dietary or surgical condition groups.

+ Different from CHOW untreated control group, $p < .05$, Newman-Keuls' post hoc t-test.

++ Different from CHOW untreated control group, $p < .01$.

There were no significant treatment or posttreatment effects of d-fenfluramine or dietary restriction on LPL activity in IBAT of rats in the DIO and CHOW conditions groups ($F(4,51) = 1.00, p < .25$).

Table 32. Treatment and Posttreatment Effects of d-Fenfluramine or Dietary Restriction on LPL Activity in Interscapular Brown Adipose Tissue

Treatment	DIO	CHOW
Control	.17 ± .04+	.10 ± .01
D-FEN(4wk)	.09 ± .01	.12 ± .02
75% Diet	.09 ± .02	.09 ± .01
D-FEN+2wk	.18 ± .05	.09 ± .02
75% Diet+2wk	.08 ± .01	.10 ± .01

Shown are group mean ± S.E.M. lipoprotein lipase (LPL) activity in brown adipose tissue expressed as micromoles FFA/hour/milligram protein at the end of 4 weeks of d-fenfluramine treatment (D-FEN) or dietary restriction (75% Diet); after 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period (D-FEN + 2 wk) or dietary restriction followed by a 2-week ad libitum feeding period (75% Diet + 2 wk) and untreated control values from the DIO and CHOW condition group.

+ Different from untreated CHOW control group, $p < .01$, Newman

Kuel's post hoc t-test.

Plasma insulin concentrations. Plasma insulin concentrations, expressed as microunits insulin/milliliter plasma, of rats treated with d-fenfluramine or a restricted diet for 4 weeks and untreated control rats from the OVX, DIO and CHOW dietary or surgical condition groups are shown in Figure 23. Plasma samples were obtained from trunk blood collected between 08:00 and 14:00 hours immediately after decapitation of rats fasted overnight (12-18 hours).

Plasma insulin concentrations in untreated control rats from the OVX and DIO groups were elevated significantly compared to insulin concentrations in control rats from the CHOW group and insulin concentrations of rats from the DIO group were slightly, but significantly, less than insulin concentrations of rats from the OVX and group (2-way ANOVA, $F(2,44) = 45.58$, $p < .001$).

There was no significant effect of d-fenfluramine treatment or dietary restriction for 4 weeks on plasma insulin concentrations ($F(2,44) = 0.99$, $p > .25$).

Plasma insulin concentrations of d-fenfluramine-treated rats and diet-restricted rats in the DIO and CHOW condition groups that were killed at the end of the treatment period or after a 2-week posttreatment period are shown in Figure 24. Plasma insulin concentrations of rats in the DIO condition group were significantly higher than plasma insulin concentrations of rats in the CHOW group ($F(1,50) = 56.38$, $p < .001$). There was no significant effect of d-fenfluramine treatment or of dietary restriction on plasma insulin concentrations ($F(4,50) = 1.01$, $p > .25$).

Plasma glucose concentrations. Plasma glucose concentrations, expressed as milligrams glucose/deciliter plasma, of d-fenfluramine-treated rats or diet-restricted rats killed at the end of the 4-week treatment period and untreated control rats from the

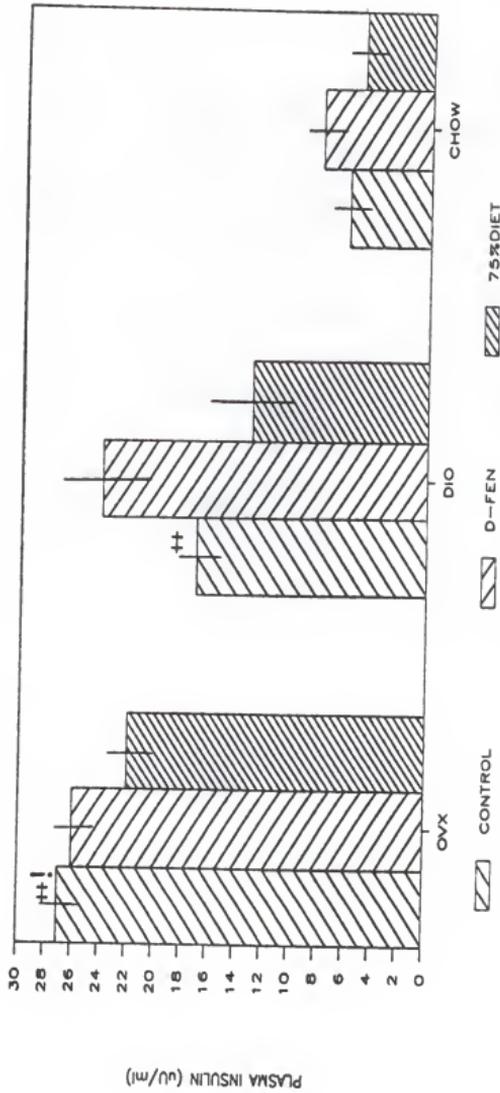


Figure 23. Effects of chronic (4-week) d-fenfluramine treatment or dietary restriction on plasma insulin concentrations. Plasma insulin concentrations, expressed in microunits insulin/milliliter plasma ($\mu\text{U}/\text{ml}$), are shown for d-fenfluramine-treated rats (D-FEN) or diet-restricted rats (75% DIET) killed at the end of the 4-week treatment period and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.

! Different from DIO group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

++ Different from CHOW group, untreated control, $p < .01$.

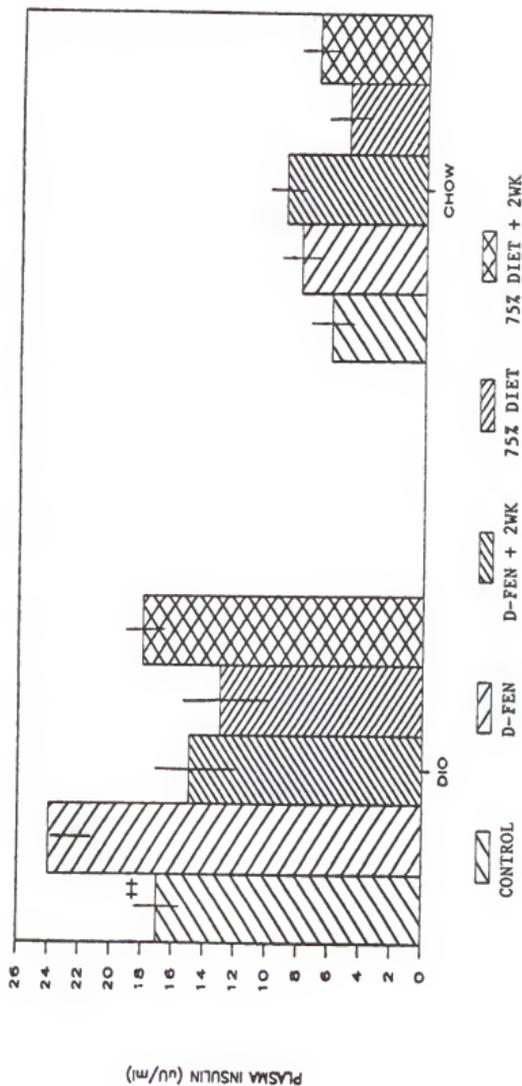


Figure 24. Treatment and posttreatment effects of chronic (4-week) d-fenfluramine or dietary restriction on plasma insulin concentrations. Plasma insulin concentrations, expressed in micro-units insulin/milliliter plasma (u/ml), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK); diet-restricted rats killed at the end of the 4-week treatment period (75% DIET) or after a 2-week ad libitum feeding period (75% DIET + 2WK) and untreated control values for rats from the DIO and CHOW dietary or surgical condition groups.

++ Different from CHOW group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

OVX, DIO and CHOW dietary or surgical condition groups are shown in Figure 25. Aliquots for glucose analyses were taken from the same plasma samples that were used for measurement of plasma insulin concentrations.

Plasma glucose concentrations of untreated control rats in the DIO condition group were significantly lower than glucose concentrations of untreated control rats in the OVX and CHOW condition groups ($F(2,50) = 3.01, p = .03$). Rats from the DIO group that received d-fenfluramine or were diet-restricted for 4 weeks had significantly higher plasma glucose concentrations compared to untreated control rats from the DIO group ($F(2,50) = 20.35, p < .001$), but plasma glucose concentrations of d-fenfluramine-treated or diet-restricted rats from the other dietary or surgical conditions were not significantly different from control rats in the corresponding condition groups.

Plasma glucose concentrations of d-fenfluramine-treated rats and diet-restricted rats killed at the end of the treatment period or after a 2-week posttreatment period are shown in Figure 26. Plasma glucose concentrations of diet-restricted or d-fenfluramine-treated rats in the DIO group that were killed after a 2-week posttreatment period were significantly lower than glucose concentrations of rats killed at the end of the treatment period ($F(4,56) = 10.05, p < .001$).

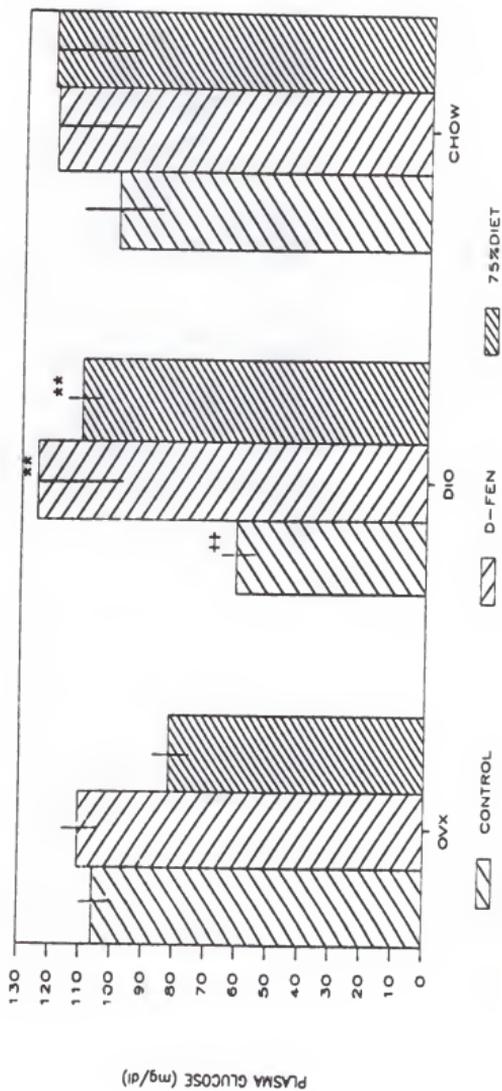


Figure 25. Effects of chronic (4-week) d-fenfluramine treatment or dietary restriction on plasma glucose concentrations. Plasma glucose concentrations, expressed in milligrams glucose/deciliter plasma (mg/dl), are shown for d-fenfluramine-treated rats (D-FEN) or diet-restricted rats (75% DIET) killed at the end of the 4-week treatment period and untreated control values for rats from the Ovx, DIO and CHOW dietary or surgical condition groups.

** Different from corresponding group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.
 ++ Different from CHOW group, untreated control, $p < .01$.

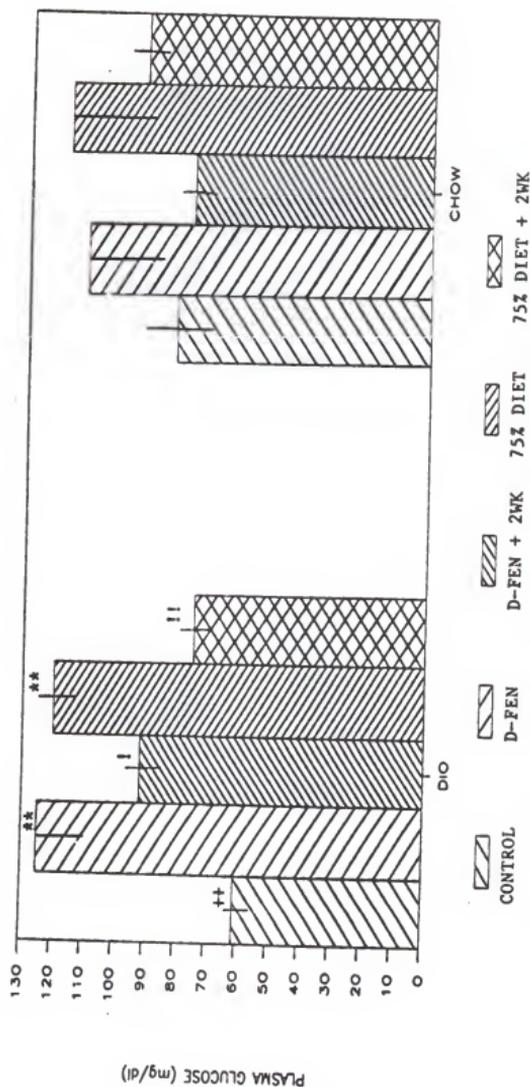


Figure 26. Treatment and posttreatment effects of chronic (4-week) d-fenfluramine or dietary restriction on plasma glucose concentrations. Plasma glucose concentrations, expressed in milligrams glucose/deciliter plasma (mg/dl), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK); diet-restricted rats killed at the end of the 4-week treatment period (75% DIET) or after a 2-week ad libitum feeding period (75% DIET + 2WK) and untreated control values for rats from the DIO and CHOW dietary or surgical condition groups.

** Different from corresponding group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

! Different from corresponding D-FEN group, $p < .01$.

!! Different from corresponding 75% DIET group, $p < .01$.

++ Different from CHOW group, untreated control, $p < .01$.

Plasma triglyceride concentrations. Plasma triglyceride concentrations, expressed as milligrams triglyceride/deciliter plasma, of d-fenfluramine-treated rats and diet-restricted rats killed at the end of the 4-week treatment period and untreated control rats from the OVX, DIO and CHOW dietary or surgical condition groups are shown in Figure 27. Triglyceride concentrations were measured in aliquots of the same plasma samples that were used for the preceding assays.

Plasma triglyceride concentrations were not significantly different among the condition groups (2-way ANOVA, $F(2,43) = 2.28$, $p = .08$). Neither dietary restriction nor d-fenfluramine treatment had a significant effect on plasma triglyceride concentrations ($F(2,43) = 2.22$, $p = .08$).

Plasma triglyceride concentrations of d-fenfluramine-treated rats or diet-restricted rats killed at the end of the treatment period or after a 2-week drug-free period with ad libitum feeding and untreated control rats from the DIO and CHOW condition groups are shown in Figure 28. The differences in plasma triglyceride concentrations among the treatment groups in the DIO and CHOW conditions were not statistically significant ($F(4,48) = 1.17$, $p > .25$).

Plasma corticosterone concentrations. Plasma corticosterone concentrations, expressed as micrograms corticosterone/deciliter plasma, of d-fenfluramine-treated rats and diet-restricted rats killed at the end of the 4-week treatment period and untreated

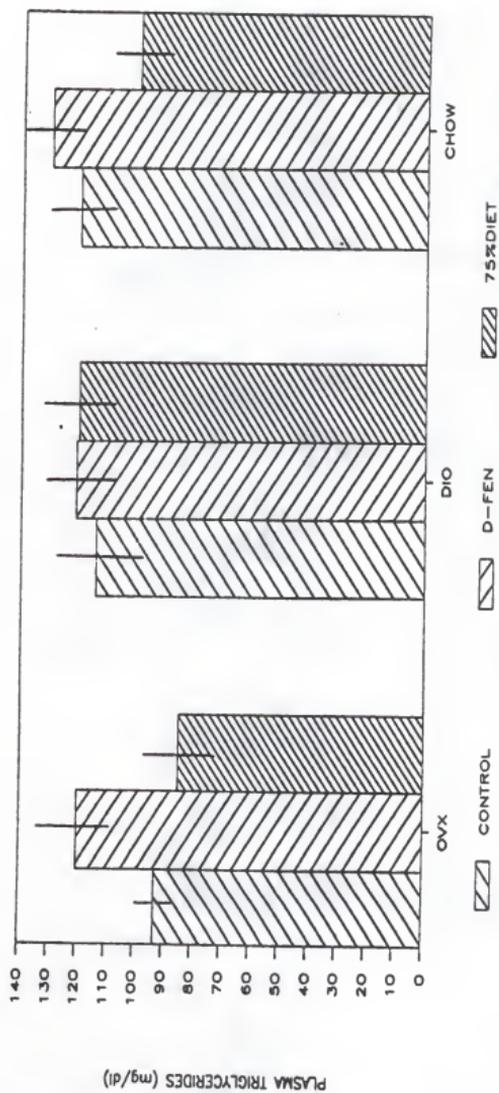


Figure 27. Effects of chronic (4-week) d-fenfluramine treatment or dietary restriction on plasma triglyceride concentrations. Plasma triglyceride concentrations, expressed in milligrams triglyceride/deciliter plasma (mg/dl), are shown for d-fenfluramine-treated rats (D-FEN) or diet-restricted rats (75% DIET) killed at the end of the 4-week treatment period and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.

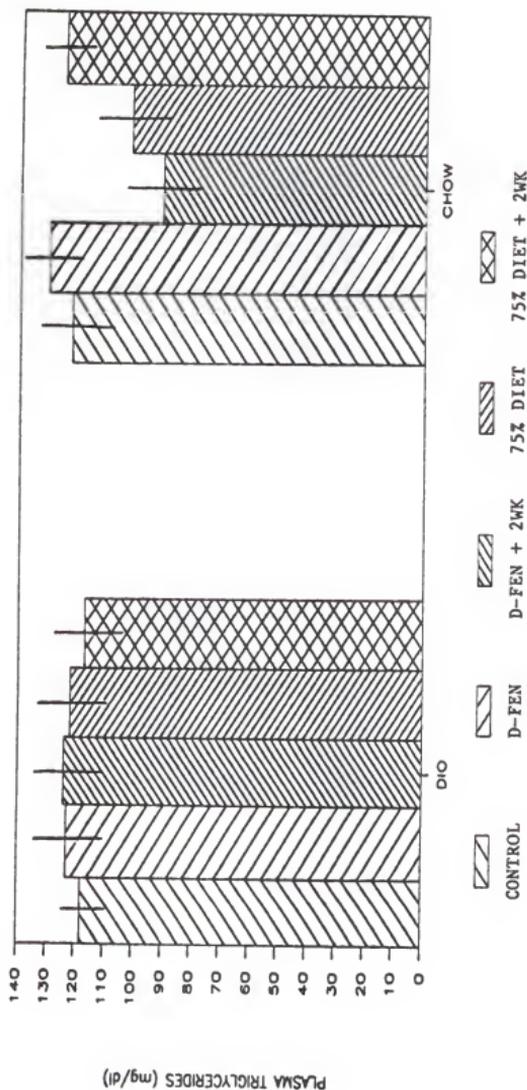


Figure 28. Treatment and posttreatment effects of chronic (4-week) d-fenfluramine or dietary restriction on plasma triglyceride concentrations. Plasma triglyceride concentrations, expressed in milligrams triglyceride/deciliter plasma (mg/dl), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK); diet-restricted rats killed at the end of the 4-week treatment period (75% DIET) or after a 2-week ad libitum feeding period (75% DIET + 2WK) and untreated control values for rats from the DIO and CHOW dietary or surgical condition groups.

control rats from the OVX, DIO and CHOW dietary or surgical condition groups are shown in Figure 29. Corticosterone concentrations were measured in aliquots of the same plasma samples that were used for the preceding assays.

Plasma corticosterone concentrations were elevated significantly in untreated control rats from the DIO group, but were not significantly different in control rats from the OVX group compared to control rats in the CHOW group (2-way ANOVA, $F(2,51) = 10.69$, $p < .001$). Dietary restriction or administration of d-fenfluramine for 4 weeks produced significant elevations in plasma corticosterone concentrations in rats from all three dietary or surgical condition groups compared to the corresponding group controls ($F(2,51) = 19.11$, $p < .001$). There was no significant interaction between the condition and treatment variables ($F(4,51) < 1.00$, $p > .25$).

Plasma corticosterone concentrations of d-fenfluramine-treated rats or diet-restricted rats killed after at the end of the treatment period or after a 2-week drug-free period with ad libitum feeding and untreated control rats from the DIO and CHOW condition groups are shown in Figure 30. By 2 weeks after the termination of d-fenfluramine treatment or dietary restriction, plasma corticosterone concentrations had returned to control levels in rats from the DIO and CHOW groups. Plasma corticosterone concentrations of d-fenfluramine-treated rats or diet-restricted rats that were killed after the 2-week posttreatment

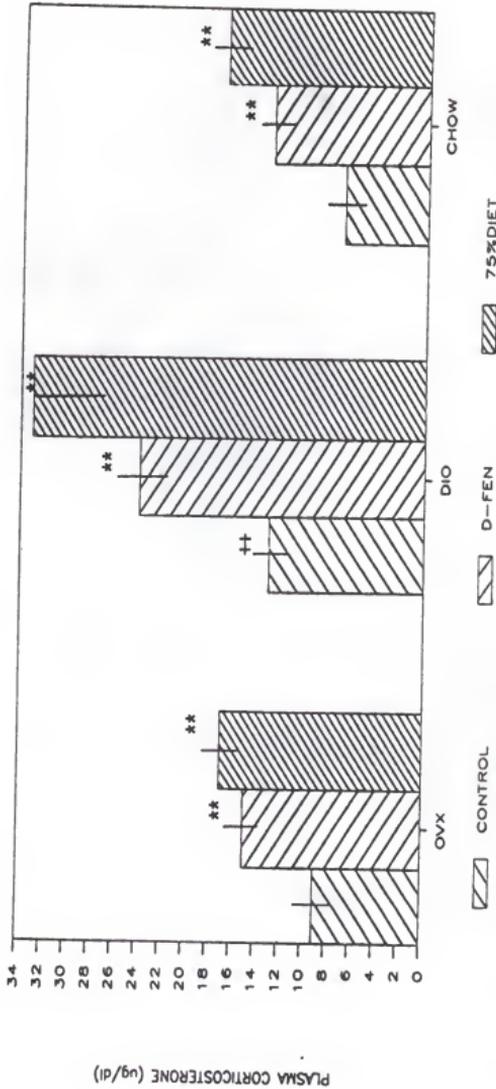


Figure 29. Effects of chronic (4-week) d-fenfluramine treatment or dietary restriction on plasma corticosterone concentrations. Plasma corticosterone concentrations, expressed in micrograms corticosterone/deciliter plasma (ug/dl), are shown for d-fenfluramine-treated rats (D-FEN) or diet-restricted rats (75% DIET) killed at the end of the 4-week treatment period and untreated control values for rats from the OVX, DIO and CHOW dietary or surgical condition groups.
 ** Different from corresponding group, untreated control, $p < 0.01$, Newman-Kuel's post hoc t-test.
 ++ Different from CHOW group, untreated control, $p < 0.01$.

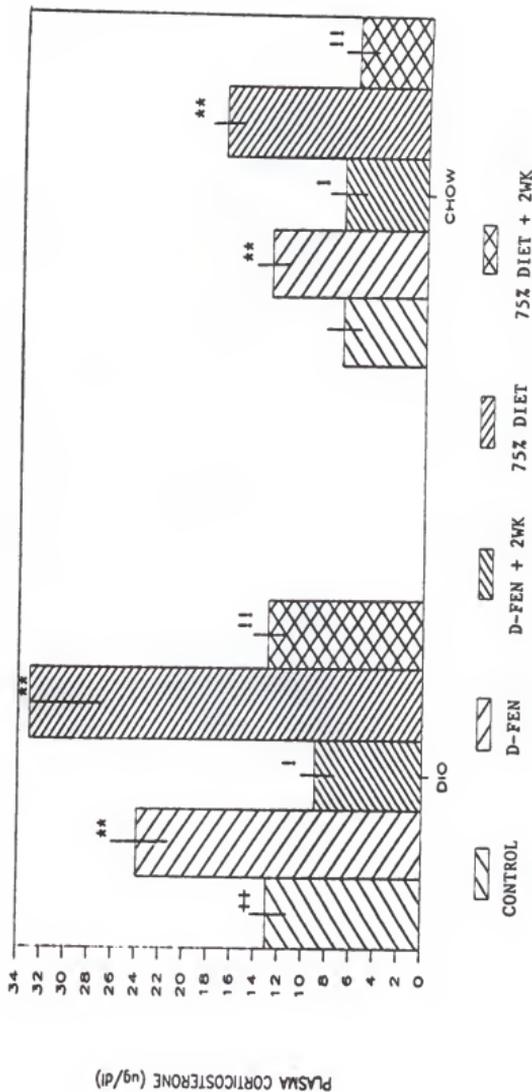


Figure 30. Treatment and posttreatment effects of chronic (4-week) d-fenfluramine or dietary restriction on plasma corticosterone concentrations. Plasma corticosterone concentrations, expressed in micrograms corticosterone/deciliter plasma (ug/dl), are shown for d-fenfluramine-treated rats killed at the end of the 4-week treatment period (D-FEN) or after a 2-week drug-free posttreatment period (D-FEN + 2WK); diet-restricted rats killed at the end of the 4-week treatment period (75% DIET) or after a 2-week ad libitum feeding period (75% DIET + 2WK) and untreated control values for rats from the DIO and CHOW dietary or surgical condition groups.

** Different from corresponding group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

! Different from corresponding D-FEN group, $p < .01$.

!! Different from corresponding 75% DIET group, $p < .01$.

++ Different from CHOW group, untreated control, $p < .01$.

period were significantly lower than corticosterone concentrations of rats killed at the end of the drug treatment period ($F(4,57) = 15.83, p < .001$). The interaction between the treatment and dietary or surgical group variables was not significant ($F(4,57) = 1.62, p = .18$).

Experiment 2: Discussion

Effects of d-Fenfluramine or Dietary Restriction on Food Intake and Body Weight

The effects of 4 weeks of administration of 3 mg d-fenfluramine/kg/day were compared to the effects of 4 weeks of dietary restriction in obese ovariectomized (OVX), dietary obese (DIO) and unoperated Chow-fed (CHOW) rats. Fenfluramine was administered continuously via osmotic minipump throughout the 4-week treatment period. The rats in the dietary restriction groups in each of OVX, DIO and CHOW dietary or surgical conditions received no drug treatment, but were given access to only 75% of their baseline daily food intakes during the 4-week treatment period. The purpose of this experiment was to compare the effects of d-fenfluramine administration to a 25% dietary restriction in the absence of drug treatment.

The decision to use a 25% dietary restriction was based on a preliminary experiment in which the mean food intake of dietary obese rats was reduced to approximately 75% of baseline by this d-fenfluramine treatment regimen. In the present experiment, the mean 2-day caloric intakes of dietary obese rats, analyzed at

specific days during the treatment period, was approximately 75% of baseline through the second week of treatment; however, the 28-day mean caloric intake in the present study was 86% of baseline and in d-fenfluramine-treated rats from the other two condition groups, food intake was not significantly different from untreated control values.

Measurements of food intake, body weight and food efficiency in rats from the DIO and CHOW condition groups were continued for a 2-week drug-free posttreatment period during which diet-restricted rats were returned to ad libitum feeding conditions. The rats in the d-fenfluramine-treated and untreated control groups were the same as in Experiment 1. The purpose of this experiment was to compare the posttreatment effects of d-fenfluramine and dietary restriction in lean and obese rats.

During the 2-week posttreatment period, food intakes of both Chow-fed and dietary obese rats that had been on a restricted diet or had received d-fenfluramine were not significantly different from the food intakes of rats that had received no treatment.

Administration of d-fenfluramine for 4 weeks resulted in a weight loss in ovariectomized rats and significantly reduced weight gain in dietary obese and Chow-fed unoperated rats. This treatment regimen was equally as effective as 25% caloric restriction in dietary obese rats, but was less effective in reducing body weights of obese ovariectomized and unoperated Chow-fed

rats. Dietary restriction produced weight loss in the ovariectomized and unoperated Chow-fed rats and significantly reduced weight gain in dietary obese rats compared to untreated control rats in the corresponding groups. This is not surprising since d-fenfluramine failed reduce food intakes by 25% throughout the experiment. Because of this, it is not possible to make definitive conclusions on proposed antiobesity actions of d-fenfluramine that may occur independently of decreased food intake and body weight.

The difference in the effectiveness of the two procedures was most pronounced in ovariectomized rats. The body weights of obese ovariectomized rats were decreased both by dietary restriction procedures and by d-fenfluramine treatment to a greater extent than rats in the other dietary or surgical condition groups. The excessive weight loss in ovariectomized rats on a restricted diet was not expected. Ovariectomized rats have been reported to gain weight when pair-fed to ovariectomized estradiol-treated rats and the obesity that develops following ovariectomy is not dependent upon hyperphagia (Roy and Wade, 1977). The time interval following ovariectomy may be an important factor as discussed in Experiment 1. Ovariectomized rats show an initial dynamic phase of weight gain that lasts about 1 month and then levels off to a trajectory similar to control rats (Wade and Gray, 1979). Thus during the later "plateau" phase of obesity, ovariectomized rats may be more sensitive to weight reduction procedures.

During the posttreatment interval, rats that had received d-fenfluramine or dietary restriction gained weight more rapidly than their controls even though their daily mean food intake was not significantly increased relative to controls. The increased weight gain of previously restricted rats was accompanied by a marked (94-123%) increase in food efficiency. This dramatic increase in food efficiency in rats that were recovering from dietary restriction is consistent with previously reported increases in food efficiency and weight gain following episodes of experimentally imposed weight loss (Coscina and Dixon, 1983; Pertschuk et al., 1983; Rolls et al., 1980).

As noted in Experiment 1, the failure to find a significant effect of d-fenfluramine on food efficiency is curious. During d-fenfluramine treatment, unoperated Chow-fed rats gained less weight than untreated group controls, but did not have significantly lower food intakes. During the posttreatment period, the rate of weight gain of rats in both the DIO and CHOW condition groups was increased relative to untreated control rats in those condition group, but again food intake was not significantly affected. Thus food efficiency estimated from [weight gain/kilocalorie ingested] may not be a reliable index in these situations.

Effects of d-Fenfluramine or Dietary Restriction on Adipocyte Size

The mean diameters of inguinal adipocytes of ovariectomized and dietary obese and unoperated Chow-fed rats were similar. Both dietary restriction and d-fenfluramine treatment reduced mean inguinal adipocyte diameter in all three condition groups. The mean adipocyte diameter in d-fenfluramine-treated rats in the DIO and CHOW groups was less than that of rats in those groups that had received dietary restriction, but the adipocyte diameter of diet-restricted rats in the OVX group was less than that of d-fenfluramine-treated rats in that group.

Both d-fenfluramine and dietary restriction reduced inguinal white adipocyte size in all three groups of rats. Two weeks after the return to ad libitum feeding the diameters of inguinal adipocytes of dietary obese and unoperated Chow-fed rats that had been diet-restricted had not returned to control levels. The inguinal adipocyte diameters of d-fenfluramine-treated rats killed after a 2-week drug-free period had partially recovered, but remained significantly greater than adipocyte diameters of rats killed at the end of the treatment period.

As discussed in Experiment 1, the similarity in adipocyte diameters of untreated control rats in the obese OVX and DIO condition groups and the CHOW group indicates that the increased adiposity of obese ovariectomized and dietary obese rats in this study reflects adipocyte hyperplasia since there is no evident

hypertrophy. The time course for development of adipocyte hyperplasia appears to be of critical importance in assessing the long-term effects of overfeeding and weight changes subsequent to changes in diet. Overfeeding for a period of time that is sufficient to allow hyperplasia to develop appears to have long-lasting or permanent effects on body weight. In humans or experimental animals with increased adipocyte number, dietary restriction or anorectic drug treatment may decrease the amount of lipid stored in existing adipocytes; however, when storage is reduced to a given level, further reductions become increasingly difficult. When dietary restriction is eased, or drug treatment terminated, adipocytes will not have decreased in number and may simply refill with time. In the present experiment, inguinal adipocyte diameters of d-fenfluramine-treated rats had partially recovered toward untreated control levels, but remained low in diet-restricted rats. Perhaps the 2-week posttreatment period did not allow sufficient time to see more complete reversals of adipocyte size.

Effects of d-Fenfluramine on Brain 5-HT and DA

The effects of d-fenfluramine treatment on concentrations of 5-HT, 5-HIAA, DA and DOPAC in rat telencephalon and hypothalamus were compared to dietary restriction and untreated control values. Neither continuous delivery of d-fenfluramine by osmotic minipump nor dietary restriction for 4 weeks affected the

concentration of 5-HT in the telencephalon. Dietary restriction produced a slight, but statistically significant, decrease in the concentration of 5-HIAA and in the ratio of 5-HIAA/5-HT in the telencephalon. Fenfluramine treatment, but not dietary restriction, produced a significant increase in the concentration of 5-HT in the hypothalamus, but the concentration of 5-HIAA and the ratio of 5-HIAA/5-HT were not affected by either treatment. In Experiment 1, the concentration of 5-HIAA in the hypothalamus was reported to be decreased. The reason for the differences in the reported results between the two experiments is the difference in statistical tests used. In Experiment 1, the concentration of 5-HIAA in d-fenfluramine-treated rats was compared to that of untreated controls by Student's t-test. In the present study, the concentration of 5-HIAA was compared in d-fenfluramine-treated, diet-restricted and untreated control rats by 1-way ANOVA. The difference in 5-HIAA concentrations in d-fenfluramine-treated versus untreated control rats was slight and the practical significance of this difference is questionable.

The effects of 4 weeks of d-fenfluramine treatment followed by a 2-week drug-free period or dietary restriction followed by a 2-week ad libitum feeding period on brain 5-HT were compared to those measured at the end of the treatment period and to untreated controls. In the telencephalon, there were no significant differences among the treatment groups in the concentrations of 5-HT,

but the concentration 5-HIAA was lower in rats killed at the end of 4 weeks of dietary restriction than in the other treatment groups. The effects of dietary restriction or d-fenfluramine treatment were reversed by the end of the 2-week posttreatment period except that the ratio of 5-HIAA/5-HT in the telencephalon of rats that were diet-restricted remained low after 2-weeks of ad libitum feeding.

The decrease in 5-HIAA/5-HT seen with dietary restriction is consistent with the proposed role of 5-HT as a mediator of satiety (e.g. Blundell, 1979): thus "hungry" animals would have lowered 5-HT activity. The majority of previous studies on the role of brain 5-HT and feeding have utilized procedures that alter brain 5-HT concentrations (either increased or decreased) and then measured effects on food intake. There is considerable variability in the results and conclusions of studies on brain 5-HT and appetite; however, the consensus appears to favor a role for 5-HT in mediating or enhancing satiety. Some, but not all procedures that decrease brain concentrations of 5-HT cause increased food intake and body weight and some, but not all, pharmacological manipulations that appear to increase brain 5-HT activity can decrease food intake and body weight (Blundell, 1979).

A second approach to the study of the relationship between brain 5-HT and appetite is to assess the effects of experimentally induced variations in food intake on brain 5-HT. These studies

have focused on the effects of specific nutrients on brain 5-HT levels and the general conclusion seems to be that under certain conditions, increased carbohydrate intake may increase brain 5-HT concentrations; however, whether or not this reflects a functional increase in 5-HT activity has been seriously questioned (Fernstrom, 1987).

Comparison of the Peripheral Actions of d-Fenfluramine to the Effects of Dietary Restriction

The results of Experiment 1 supported the hypothesis that chronic d-fenfluramine treatment is more effective in reducing body weight in obese than in lean rats. Furthermore, substantial reductions in body weight occurred in the absence of sustained suppression of food intake in obese ovariectomized and unoperated Chow-fed rats. Peripheral effects of d-fenfluramine were examined for clues to possible antiobesity actions that might function to reduce body weight independently of decreased food intake. These include (a) stimulation of ATPase activity in brown adipose tissue of ovariectomized and unoperated Chow-fed rats presumably reflecting increased thermogenesis and energy burning; (b) stimulation of muscle ATPase activity in ovariectomized rats suggesting increased muscular activity or increased cost of muscular activity and (c) substantial reductions in lipoprotein lipase activity in retroperitoneal adipose tissue of rats in all dietary or surgical condition groups implying reduced lipid storage in subcutaneous adipose tissue. In apparent contradiction

to antiobesity actions were the failure of d-fenfluramine to reduce the hyperinsulinemia found in ovariectomized, dietary obese and estradiol-treated rats and the marked hypercorticism produced by chronic d-fenfluramine administration.

In order to further examine these actions and to determine the extent to which they may be independent of reduced food intake or body weight, the peripheral effects of 4 weeks of d-fenfluramine administration via osmotic minipump were compared to the peripheral effects of 4 weeks of a restricted diet that allowed rats access to 75% of their baseline caloric intakes and these two treatments were compared to untreated control values for the ovariectomized, dietary obese and unoperated Chow-fed rats. These peripheral effects were measured in rats from the OVX, DIO and CHOW condition groups killed at the end of the 4-week drug or restricted diet treatment. Additional groups of rats in the DIO and CHOW conditions were killed after a 2-week drug-free, ad libitum feeding period.

NPPase activity. The activity of K^+ -nitrophenylphosphatase (NPPase), an indicator of Na^+-K^+ (ATPase) activity, in interscapular brown adipose tissue (IBAT) of untreated dietary obese and unoperated Chow-fed rats was 4-times greater than NPPase activity of ovariectomized rats. Administration of d-fenfluramine for 4 weeks increased NPPase activity in IBAT of ovariectomized rats to a level almost identical to that of untreated controls in

the DIO and CHOW condition groups (i.e. a 4-fold increase). Fenfluramine treatment also increased NPPase activity in IBAT of Chow-fed unoperated rats, although the increase was not as large as in ovariectomized rats. There was not a significant effect of d-fenfluramine on NPPase activity in IBAT of dietary obese rats. The dramatic increase in NPPase activity in IBAT of ovariectomized rats may be due to the low control levels of enzyme activity in these rats: because of the low control levels of NPPase activity, ovariectomized rat may have been more sensitive to d-fenfluramine-induced changes in enzyme activity. By the end of the 2-week drug-free period, NPPase activity in IBAT of rats in the CHOW condition group that had been treated with d-fenfluramine had returned to control levels. There was no significant effect of dietary restriction on the activity of NPPase in IBAT of rats killed at the end of the 4-week treatment period or of rats killed after a 2-week ad libitum feeding posttreatment period.

The low levels of NNPase activity in ovariectomized rats are consistent with reports of lowered thermogenic activity in ovariectomized rats that is dependent upon sympathetic nervous system activity and is reversed by administration of estradiol benzoate (Bartness and Wade, 1984). The d-fenfluramine-stimulated increase in IBAT thermogenesis in rats from the OVX and CHOW condition groups may be related to increased sympathetic activity since acutely administered fenfluramine has mild sympathomimetic effects

(Lake et al., 1979) that may be 5-HT mediated (Stajarne and Schapiro, 1959). In the current study, d-fenfluramine administration did not brain deplete 5-HT; therefore, the chronic treatment effects seen here may be similar to acute effects observed with higher doses.

The effects of d-fenfluramine treatment on NPPase in IBAT were consistent with its increased efficacy in producing weight loss in ovariectomized rats. Dietary restriction and weight loss have been associated with decreased thermogenesis in IBAT under some conditions. Perhaps one reason that dietary restriction was so effective in reducing body weights of ovariectomized rats in the present experiment is related to the failure to further decrease the already low NPPase activity in brown adipose tissue of ovariectomized rats. Conversely the effectiveness of d-fenfluramine treatment in reducing body weights of ovariectomized rats in the absence of decreased food intake may have been related to the stimulation of NPPase activity of IBAT.

Experimental evidence (eg. Rothwell and Stock, 1979a, 1979b), along with clinically observed variations in efficiency of metabolism, suggest that in some situations increased caloric intake is accompanied by increased energy expenditure and an increase in diet-induced thermogenesis (Danforth, 1981; Landsberg and Young, 1981; Rothwell and Stock, 1979b). In experimental animals, this has been attributed to increased metabolic activity in BAT

(Rothwell and Stock, 1979a; Stirling and Stock, 1960). Cafeteria feeding for a period of 2 weeks has been associated with hypertrophy of IBAT (Armitage et al., 1983; Himms-Hagen et al., 1981; Rothwell and Stock, 1979b) and unmasking of GDP-binding sites in IBAT mitochondria (Himms-Hagen et al., 1981). Analogous changes are seen with cold exposure (Armitage et al., 1983) and with chronic administration of NE (Desautels and Himms-Hagen, 1979; Himms-Hagen et al., 1981). The increase in IBAT weight induced by diet persists with return to Chow feeding (Tulp, 1981) whereas IBAT size returns to normal upon termination of cold exposure (Himms-Hagen et al., 1972).

Swann (1984a) reported diet-induced changes in activity of the enzyme ($\text{Na}^+\text{-K}^+$)ATPase in IBAT and muscle that appeared to be regulated by beta-adrenergic receptors. Cafeteria feeding increased ATPase activity and this activity remained elevated with return to regular diet. The rats in that experiment increased their caloric intake by 80% but did not gain weight relative to controls. Food deprivation resulted in decreased ATPase activity that persisted upon refeeding. During the period of refeeding, rats gained weight approximately 3-times faster than nondeprived controls. Certainly the results of the present experiment do not support this notion.

The activity of NPPase in gastrocnemius muscle was decreased in ovariectomized rats by approximately the same extent as enzyme

activity in IBAT (<25% of CHOW control value). The NPPase activity of dietary obese rats was also reduced although to a lesser extent than in ovariectomized rats (55% of CHOW control value). Administration of d-fenfluramine for 4 weeks increased the activity of muscle NPPase in ovariectomized rats by approximately 47%, but the increase was not sufficient to raise enzyme activity in these rats to the same level as in untreated control rats from the CHOW group.

Administration of d-fenfluramine had no significant effect on muscle NPPase activity in rats from the other dietary or surgical condition groups compared to untreated controls in those groups, thus enzyme activity in d-fenfluramine-treated dietary obese rats remained below the level of activity in unoperated Chow-fed rats. Perhaps the pronounced effects of d-fenfluramine on NPPase activity in ovariectomized rats and the relative lack of effectiveness in dietary obese rats is one of the reasons for the greater efficacy of d-fenfluramine in producing weight loss in ovariectomized rats compared to the dietary obese and other groups, although the hypophagic effects were more obvious in dietary obese rats.

Four weeks of dietary restriction decreased muscle NPPase activity substantially (20-50%) in all three dietary or surgical condition groups. Dietary restriction decreased NPPase activity whereas d-fenfluramine treatment tended to increase enzyme

activity; therefore, there was a significant interaction between the dietary or surgical condition variable and the treatment variable.

Muscle NPPase activity was significantly lower in dietary obese rats than in unoperated Chow-fed rats. Dietary restriction produced a decrease in the activity of muscle NPPase that was reversed by a 2-week period of ad libitum feeding in rats from the unoperated Chow-fed rats, but not in dietary obese rats. There was no significant effect of d-fenfluramine treatment on muscle NPPase activity of rats in dietary obese or unoperated Chow-fed rats that were killed at the end of drug treatment or after a 2-week drug-free period.

Lipoprotein lipase (LPL) activity. The activity of retroperitoneal LPL was lower in dietary obese rats and higher in ovariectomized rats than in unoperated Chow-fed control rats. Four weeks of chronic d-fenfluramine administration substantially (38-78%) reduced retroperitoneal LPL activity in rats from each of the three dietary or surgical condition groups. Dietary restriction had no significant effect on LPL activity in retroperitoneal adipose tissue.

At the end of the 2-week drug-free period, LPL activity in retroperitoneal adipose tissue of dietary obese rats that had received d-fenfluramine had returned to control levels. Lipoprotein lipase activity in retroperitoneal adipose tissue of

unoperated Chow-fed rats had been affected by d-fenfluramine treatment to a greater extent and had not returned to control levels within 2 weeks of the termination of drug treatment.

Lipoprotein lipase activity in inguinal WAT was also elevated in ovariectomized rats compared to rats in the other dietary or surgical condition groups; however, LPL activity in dietary obese rats was not significantly different from that of unoperated Chow-fed rats. Neither dietary restriction nor administration of d-fenfluramine for 4 weeks had significant effects on inguinal LPL activity in any of the dietary or surgical condition groups.

The activity of LPL in IBAT was higher in both ovariectomized and dietary obese rats than in unoperated Chow-fed rats. Neither dietary restriction nor administration of d-fenfluramine for 4 weeks had significant effects on LPL activity in IBAT and there were no significant differences in the activity of LPL in IBAT samples taken from previously d-fenfluramine-treated rats or diet-restricted rats killed after a 2-week drug-free, ad libitum feeding period compared IBAT samples taken at the termination of drug treatment or untreated controls.

In the present study, LPL activity was decreased by approximately 50% in retroperitoneal white and interscapular brown, but not in inguinal, adipose tissue of dietary obese rats compared to unoperated Chow-fed rats. Previous reports on changes in LPL activity with diet-induced obesity are inconsistent; both

increases and decreases have been reported. The differences in diet-induced changes in LPL activity in the different adipose tissues are consistent with previous reports of tissue differences in the responsivity of LPL to changes in diet.

In some situations, changes in LPL activity are associated with weight changes and with extended dieting, LPL activity may be increased, an effect that would have important implications for long term weight regulation (Schwartz and Brunzell, 1981). Lipoprotein lipase has been reported to be sensitive to changes in diet and body weight. Adipocyte LPL is related to cell size (Hietanen and Greenwood, 1977; Brunzell, 1979) and is elevated in several animal models of obesity as well as in a population of obese and formerly obese humans. The rate of weight loss in dieting humans is inversely correlated with progressive changes in LPL activity. Thus during the initial rapid weight loss that accompanies a hypocaloric diet, there is no change in LPL activity. With continued caloric restriction however, LPL activity is increased; weight loss slows and the individual often reports increasing discomfort with the restricted diet (Schwartz and Brunzell, 1981).

Formerly obese humans with stable, reduced weight have adipocyte LPL activity increases of 3-4-fold compared to activity measured before weight loss. With return to the original obese weight, LPL activity is restored to former levels (Schwartz and

Brunzell, 1981). These changes in LPL activity associated with changes in body weight may have important implications for understanding the difficulty in sustaining weight loss and preventing regain.

In the present study, 4 weeks of dietary restriction had no significant effect on LPL activity in any of the tissues examined. The correlation between changes in LPL activity and changes in food intake and body weight has not been found in universality; however, increased LPL activity might have been predicted from the profile of hyperinsulinemia and hypercorticism associated with dietary restriction in the present study.

Carbohydrate and lipid metabolism. Ovariectomized and dietary obese rats were hyperinsulinemic. There were no significant effects of dietary restriction or d-fenfluramine treatment on plasma insulin concentrations.

Overnight fasted dietary obese rats that received no treatment were hypoglycemic; however, dietary obese rats that received chronic d-fenfluramine treatment or dietary restriction had normal plasma glucose concentrations (100-125 mg/dl).

Plasma triglyceride concentrations of overnight fasted ovariectomized or dietary obese and unoperated Chow-fed rats were similar and there were no significant effects of dietary restriction or d-fenfluramine treatment on plasma triglyceride concentrations.

Plasma corticosterone concentrations were slightly, but significantly elevated in untreated dietary obese rats compared to untreated Chow-fed rats and ovariectomized rats. Dietary restriction or administration of d-fenfluramine for 4 weeks produced significant elevations in plasma corticosterone concentrations in rats from all three dietary or surgical condition groups compared to the corresponding group controls. By 2 weeks after the termination of d-fenfluramine treatment or dietary restriction, plasma corticosterone concentrations had returned to control levels.

The marked hyperinsulinemia associated with ovariectomy and dietary obesity is consistent with previous reports (eg. Triscari et al., 1985). Hyperinsulinemia is commonly found in a majority of obese animal models and in humans with longstanding obesity.

Chronic d-fenfluramine treatment or dietary restriction for 4 weeks had no significant effect on plasma insulin concentrations in any of the groups of rats in the present experiment. Both dietary restriction and d,l-fenfluramine treatment have been reported to improve insulin sensitivity. Perhaps the length of the treatment time was not sufficient for changes in plasma insulin concentrations to be manifested.

Dietary obese rats were hypoglycemic after being fasted overnight (12-18 hours) prior to the time they were killed. Four weeks of d-fenfluramine administration or dietary restriction elevated fasting glucose concentrations of dietary obese rats to

normal (100-125 mg/dl) levels, but had no significant effect on glucose concentrations of rats in the other dietary or surgical condition groups. The hypoglycemia in dietary obese rats was not expected; however, the metabolic profile in these rats was marked by hyperinsulinemia and relatively high circulating corticosterone concentrations. The combination of hyperinsulinemia and hypercorticism is found in both humans and in some animal models of longstanding obesity. Both dietary restriction and d-fenfluramine administration brought fasted plasma glucose concentrations within the normal range although both treatments were associated with continued hyperinsulinemia and actual increases in plasma corticosterone concentrations. The increased corticosterone concentrations would be expected to raise plasma glucose concentrations by stimulating gluconeogenesis. Insulin receptor sensitivity may also have been improved even though fasting plasma insulin concentrations were not obviously decreased; however, glucocorticoids can decrease insulin sensitivity and this may be the reason hyperinsulinemia was not improved by d-fenfluramine treatment or dietary restriction in the present study. Injection of ACTH or glucocorticoids can produce a syndrome of insulin resistant diabetes. Glucose intolerance is also observed in Cushing's Syndrome and in patients undergoing glucocorticoid therapy (Munck, 1971; Pupo et al., 1966). This appears to be due to a decrease in receptor affinity for insulin and similarly, adrenalectomy causes an increase in insulin receptor affinity (Kahn et al., 1978).

Experiment 3: Effects of d-Fenfluramine on Body Temperature,
Glucose Tolerance and Stress Responses to Cold Exposure

Body Temperature

The effects of d-fenfluramine on thermogenic activity in interscapular brown adipose tissue (IBAT) were discussed in Experiments 1 and 2. The activity of the enzyme NPPase was used as an index of cellular and metabolic activity. The activity of NPPase in IBAT in untreated ovariectomized rats was low compared to control rats in the other condition groups and was less than 25% of control values from the unoperated Chow-fed rats. Administration of d-fenfluramine increased NPPase activity in IBAT of ovariectomized rats to a level comparable to the other groups. Fenfluramine also increased NPPase activity in unoperated Chow-fed rats by 45% but did not significantly affect the activity of this enzyme in estradiol-treated or dietary obese rats.

The changes in activity of NPPase in IBAT were consistent with changes in body weight. The activity of NPPase was low in ovariectomized rats and was substantially increased by chronic d-fenfluramine treatment. Estradiol administration also increased NPPase activity in IBAT of ovariectomized rats, but d-fenfluramine treatment did not further increase NPPase activity in estradiol-treated rats.

It was hypothesized that significant changes in thermogenesis would be accompanied by changes in body temperature. In the present experiment, colonic temperatures of rats treated with

d-fenfluramine (3 mg/kg/day via osmotic minipump) and untreated controls were measured in rats from the four dietary or surgical condition groups: obese ovariectomized (OVX); ovariectomized, estradiol-treated (EB); dietary obese (DIO) and unoperated Chow-fed (CHOW).

Results. Body temperatures of d-fenfluramine-treated rats and untreated control rats in the four dietary or surgical condition groups are shown in Figure 31. Colonic temperatures were measured at an ambient temperature of 25 °C on the third day of drug treatment and after 4 hours exposure to cold (4 °C) on the fourth day of treatment and compared statistically by 3-way ANOVA with repeated measures. Untreated control rats in the OVX condition group had lower colonic temperatures and rats in the DIO group had higher colonic temperatures than untreated control rats in the EB and CHOW condition groups ($F(3,18) = 4.72, p = .01$). Four-hour exposure to cold (4 °C) caused a significant drop in colonic temperature of untreated control rats in the EB, DIO and CHOW condition groups ($F(1,6) = 32.95, p < .001$), but not in control rats in the OVX condition. There was no significant effect of d-fenfluramine treatment on body temperature ($F(1,6) = 4.01, p = .09$).

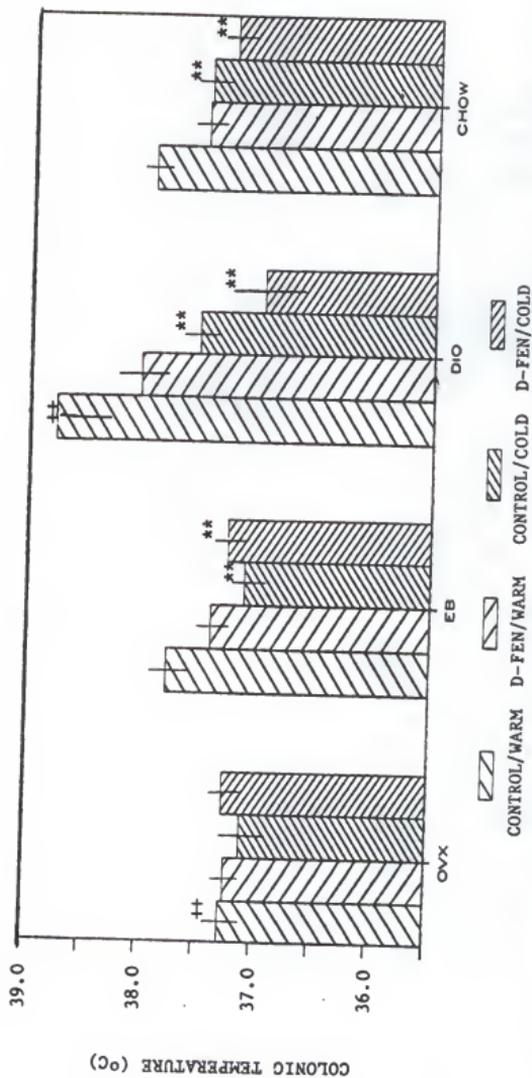


Figure 31. Effects of d-fenfluramine treatment on body temperature. Shown are colonic temperatures of d-fenfluramine-treated rats (D-FEN) and untreated control rats from the four dietary or surgical condition groups. Colonic temperatures were measured at ambient temperatures of 25 °C (WARM) on the third day of d-fenfluramine treatment and at 4 °C (COLD) on the 4th day of the treatment period. ** Different from corresponding group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test. ++ Different from CHOW group, untreated control, $p < .01$.

Discussion. Changes in thermogenesis are accompanied by changes in body temperature or in heat exchange. Levin et al. (1983) reported that rats with diet-induced obesity showed impaired thermogenic responses when exposed to cold. The lower body temperatures of ovariectomized rats in the present study were accompanied by lower levels of NPPase activity in IBAT and increased body temperatures in DIO rats were associated with increased NPPase activity in IBAT. The thermogenic response of dietary obese rats was not obviously impaired in the present experiment since during cold exposure, their colonic temperatures did not drop significantly lower than those of rats in other groups; however, the total decrease in colonic temperatures of dietary obese rats in response to cold was greater than in other groups because their temperatures were significantly higher at normal room temperature (25 °C).

In the present study, d-fenfluramine treatment tended to decrease colonic temperature, but not significantly, at either 25 °C or 4 °C. Previous reports on the effects of fenfluramine on body temperature are variable, but generally slight decreases are reported. Pawlowski et al. (1980) and Sugrue (1981, 1984) reported decreases of 1-2 °C in rectal temperatures of d,l-fenfluramine-treated rats. This effect appeared to be 5-HT dependent. It is possible that the decreases in body temperature reported in that study were due to increased heat loss since 5-HT

is a peripheral vasodilator. A similar mechanism may explain the failure to find increased body temperatures in rats that had increased thermogenic activity in IBAT.

Plasma Insulin Concentrations and Glucose Tolerance

In Experiment 1, the effects of chronic d-fenfluramine treatment on daytime plasma concentrations of glucose and insulin were measured after a 12-18 hour fast in rats from the four dietary or surgical condition groups. Obese ovariectomized rats, estradiol-treated rats and dietary obese rats were hyperinsulinemic and chronic d-fenfluramine treatment had no significant effect on plasma insulin concentrations in any of the groups of rats in that experiment. In Experiment 1, dietary obese rats were hypoglycemic after being fasted overnight (12-18 hours) prior to the time they were killed. Four weeks of d-fenfluramine administration elevated fasting glucose concentrations of dietary obese rats to normal levels, but had no significant effect on glucose concentrations of rats in the other dietary or surgical condition groups.

Although fasting plasma insulin and glucose concentrations in Experiments 1 and 2 were not affected by d-fenfluramine treatment, it was hypothesized that glucose tolerance may be a more sensitive index of the effects of d-fenfluramine on carbohydrate metabolism. Previously reported effects of d,l-fenfluramine on carbohydrate metabolism in normal weight laboratory animals and humans include increased glucose uptake (Butterfield and Whichelow, 1968)

and improved glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970).

The effects of administration of 3 mg d-fenfluramine/kg/day (via osmotic minipump) on plasma concentrations of insulin were measured under basal nighttime conditions. Plasma samples were collected from chronically indwelling jugular catheters under basal conditions on the third day of d-fenfluramine administration. Plasma insulin concentrations of d-fenfluramine-treated rats and untreated control rats in the four dietary or surgical condition groups were compared by 2-way ANOVA.

Results. Plasma insulin concentrations of untreated control rats in the OVX, EB and DIO dietary or surgical condition groups (Figure 32) were significantly higher than insulin concentrations of control rats in the CHOW condition group ($F(3,46) = 10.06$, $p < .001$). There was no significant effect of d-fenfluramine treatment on plasma insulin concentrations ($F(1,46) < 1.00$, $p > .25$).

Glucose tolerance was measured in 4-hour fasted d-fenfluramine-treated rats and untreated control rats from the four dietary or surgical condition groups. Glucose tolerance (Figure 33) was estimated from plasma glucose concentration measured at 20 minutes after a 25% glucose load. The glucose was administered and blood samples collected via chronically indwelling jugular catheters during the dark cycle on the third day of chronic d-fenfluramine treatment.

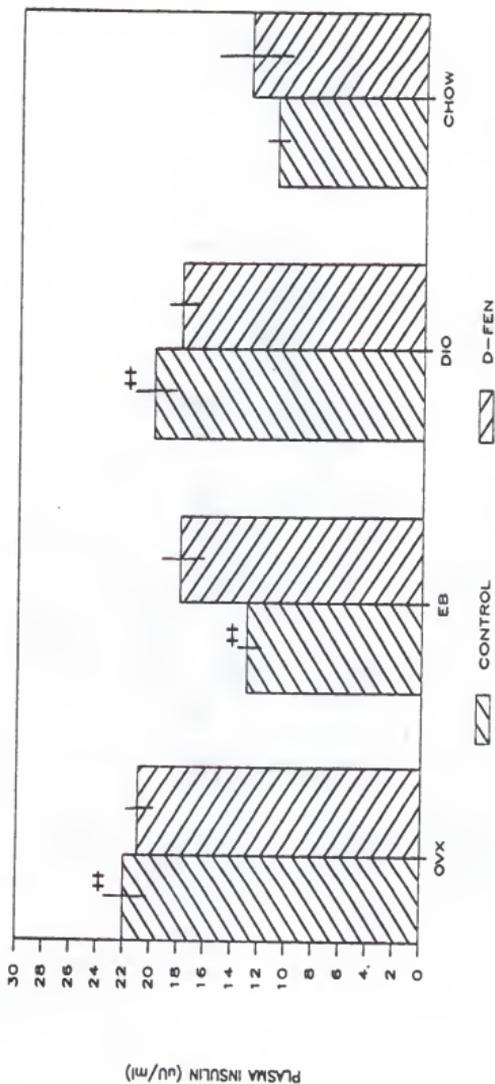


Figure 32. Effects of d-fenfluramine treatment on nighttime plasma insulin concentrations. Plasma insulin concentrations, expressed in microunits insulin/milliliter plasma ($\mu\text{U}/\text{ml}$), are shown for d-fenfluramine-treated rats (D-FEN) and untreated control rats from the four dietary or surgical condition groups. Insulin concentrations were measured on the third day of d-fenfluramine administration.

++ Different from CHOW group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

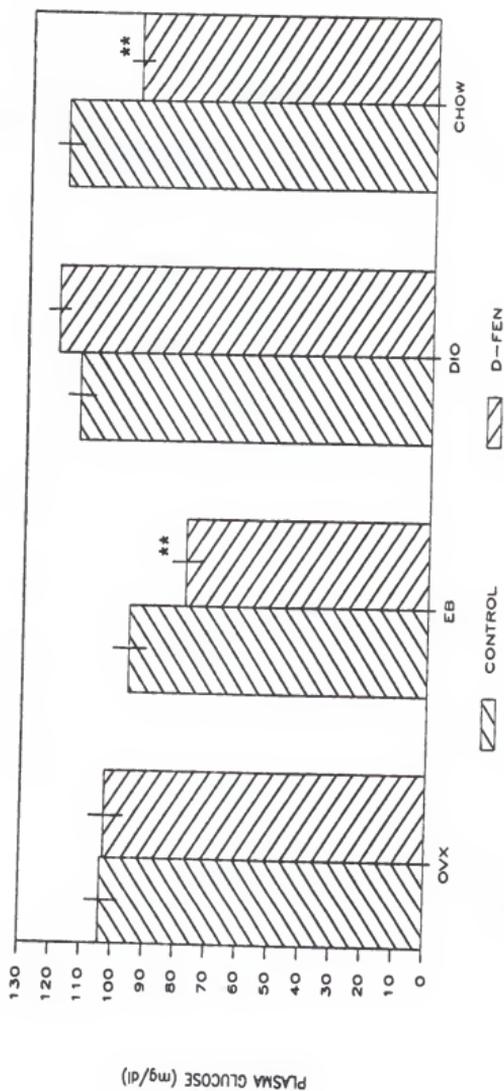


Figure 33. Effects of d-fenfluramine treatment on nighttime glucose tolerance. Plasma glucose concentrations, expressed as milligrams glucose/deciliter plasma were measured at 20 minutes after a 25% glucose load in d-fenfluramine-treated rats (D-FEN) and untreated controls from the four dietary or surgical condition groups. Glucose tolerance was measured on the third day of d-fenfluramine administration.

** Different from corresponding group, untreated control, $p < .01$, Newman-Kuel's post hoc t-test.

There were no significant differences in plasma glucose concentrations (measured at 20 minutes after a 25% glucose load) among the four dietary or surgical condition groups (2-way ANOVA, $F(3,47) = 3.33$, $p = .08$). Administration of d-fenfluramine significantly improved glucose tolerance in rats from the EB and CHOW condition groups, but not in rats from the OVX and DIO conditions ($F(1,47) = 4.19$, $p = .05$).

Discussion. In Experiments 1 and 2, obese ovariectomized rats, estradiol-treated rats and dietary obese rats were hyperinsulinemic and neither chronic d-fenfluramine treatment nor dietary restriction had significant effects on fasted, daytime plasma insulin concentrations in any of the groups of rats in those experiments. The results of the present experiment confirm those of Experiments 1 and 2. Nighttime insulin levels were elevated in ovariectomized rats, estradiol-treated rats and dietary obese rats. In Experiments 1 and 2, insulin concentrations of dietary obese rats were slightly, but significantly, less than those of obese ovariectomized and estradiol-treated rats; however insulin concentrations were not significantly different among those groups in Experiment 3.

In Experiments 1 and 2, dietary obese rats were hypoglycemic after being fasted overnight (12-18 hours) prior to the time they were killed. Four weeks of d-fenfluramine administration or dietary restriction elevated fasting glucose concentrations of

dietary obese rats to normal levels, but had no significant effect on glucose concentrations of rats in the other dietary or surgical condition groups.

In the present experiment, basal nighttime glucose concentrations of 4-hour fasted dietary obese rats tended to be higher, although not significantly ($p = .08$), than plasma glucose concentrations of rats in the other dietary or surgical condition groups. This suggests that the hypoglycemia seen in Experiments 1 and 2 was the result of a reaction to the overnight fast (12-18 hours).

Although fasting plasma glucose concentrations (Experiments 1 and 2) were not affected by d-fenfluramine treatment, in the present experiment, glucose tolerance was improved by d-fenfluramine treatment in estradiol-treated rats and unoperated Chow-fed rats but not in ovariectomized or dietary obese rats. It is possible that with continued administration of d-fenfluramine, glucose tolerance would have improved in the obese rats in this study. The improvement in glucose tolerance seen with d-fenfluramine treatment is consistent with previously reported effects of clinical doses of d,l-fenfluramine (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970).

Previously reported effects of d,l-fenfluramine on carbohydrate metabolism in normal weight laboratory animals and humans include increased glucose uptake (Butterfield and Whichelow, 1968)

and improved glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970). The insulin-dependent uptake of glucose in muscle is stimulated by d,l-fenfluramine and this appears to be mediated by 5-HT (Kirby and Turner, 1976; Turner, 1979; Turner et al., 1982). Racemic fenfluramine also has been reported to reduce fasting plasma glucose levels and improve glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970); effects that are not mediated by increased insulin release (Pasquine and Thenen, 1981).

Effects of d-Fenfluramine on Responses to Four-Hour Cold Exposure

There is indirect evidence that fenfluramine has effects on stress hormones that may be related to its antiobesity actions: (a) administration of d-fenfluramine decreases the ethanol-induced rise in plasma corticosterone (Brindley et al., 1979); (b) d,l-fenfluramine is effective in reversing obesity resulting from overeating induced by either tail pinch (Antelman et al., 1979) or central administration of muscimol (Borsini et al., 1982) both of which are proposed animal models of stress-induced eating; (c) glucocorticoid-induced obesity in humans is responsive to d,l-fenfluramine treatment (Cameron et al., 1972; Tomlinson et al., 1975) and (d) d,l-fenfluramine may be effective in treating obesity associated with stress-induced eating (Antelman and Caggiola, 1979; Robinson et al., 1985). Acutely, d-fenfluramine stimulates glucocorticoid release and chronically administered

d-fenfluramine decreases the stress-induced peak in corticosterone, but only at a very high dose (25 mg/kg) and not at lower doses (Brindley et al., 1985).

The effects of chronic administration of 3 mg d-fenfluramine/kg/day (via osmotic minipump) on plasma concentrations of triglycerides and corticosterone were measured under basal nighttime conditions and after 4 hours exposure to cold (4 °C). Plasma samples were collected from chronically indwelling jugular catheters under basal conditions on the third day of d-fenfluramine administration and after 4 hours of cold exposure on the fourth day of drug treatment.

Plasma triglycerides. Nighttime plasma triglycerides concentrations were measured in d-fenfluramine-treated rats and untreated control rats from the four dietary or surgical condition groups at 25 °C and at 4 °C (Figure 34). The data were analyzed by 3-way ANOVA with repeated measures design. Plasma triglycerides concentrations were elevated by cold exposure ($F(1,5) = 46.72, p = .001$) in all dietary or surgical condition groups. There were no significant differences in triglyceride concentrations among the condition groups ($F(3,15) < 1.00, p > .25$) and there was no significant effect of d-fenfluramine treatment on triglyceride concentrations ($F(1,5) < 1.00, p > .25$).

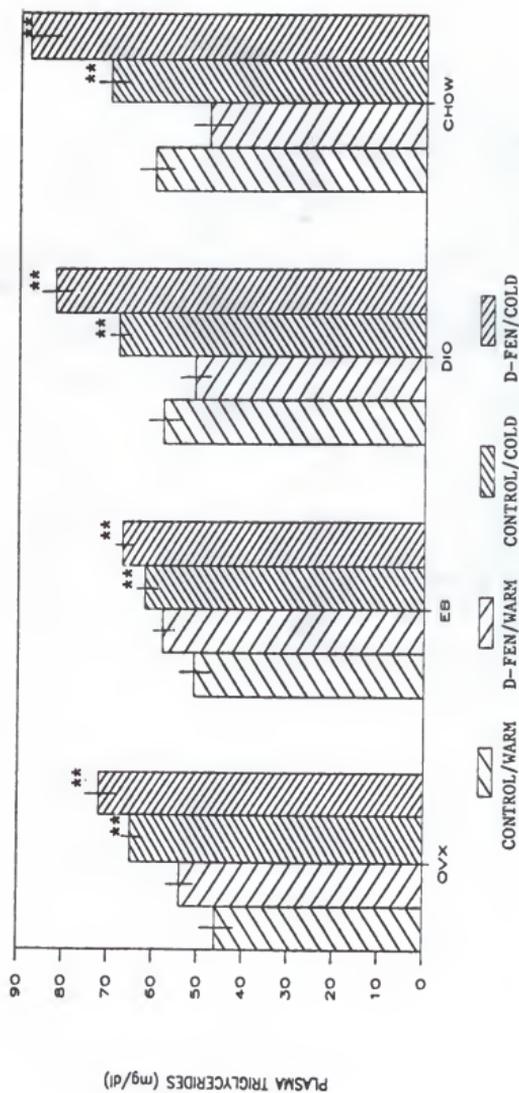


Figure 34. Effects of d-fenfluramine treatment on basal nighttime triglyceride concentrations and on the stress-induced rise in plasma triglycerides. Plasma triglyceride concentrations were measured under basal nighttime conditions (25 °C: WARM) on the third day of d-fenfluramine administration and after 4 hours of cold exposure (4 °C: COLD) on the fourth day of d-fenfluramine administration. Plasma triglyceride concentrations are expressed in milligrams triglyceride/deciliter plasma for d-fenfluramine-treated rats (D-FEN) and untreated controls in the four dietary or surgical condition groups.
 ** Different from corresponding group, untreated control at 25 °C, $p < 0.01$, Newman-Kuel's post hoc t-test.

Plasma corticosterone. Nighttime plasma corticosterone concentrations were measured in d-fenfluramine-treated rats and untreated control rats from the four dietary or surgical condition groups at 25 °C and at 4 °C (Figure 35). The data were analyzed by 3-way ANOVA with repeated measures design. Plasma corticosterone concentrations were elevated in untreated control rats from the DIO condition group compared to untreated control rats in the other dietary or surgical condition groups ($F(3,15) = 3.87, p = .03$). Plasma corticosterone concentrations were elevated by cold exposure ($F(1,5) = 19.41, p = .007$) in all dietary or surgical condition groups. Fenfluramine treatment elevated corticosterone levels in all condition groups ($F(1,5) = 7.29, p = .04$) at 25 °C, but not at 4 °C. The interaction between the treatment and temperature variables was significant ($F(1,5) = 6.88, p = .05$).

Discussion. In Experiments 1 and 2, fasted plasma triglyceride concentrations were not significantly different among untreated control rats from the four dietary or surgical condition groups and there was no significant effect of the 4-week d-fenfluramine treatment regimen on plasma triglyceride concentrations. It has been reported that d,l-fenfluramine is similarly ineffective in altering fasting triglyceride concentrations (Bizzi et al., 1973; Curtis-Prior, 1980, Garattini et al., 1975).

In the present experiment, there was a cold-induced rise in plasma triglycerides; however, the d-fenfluramine treatment

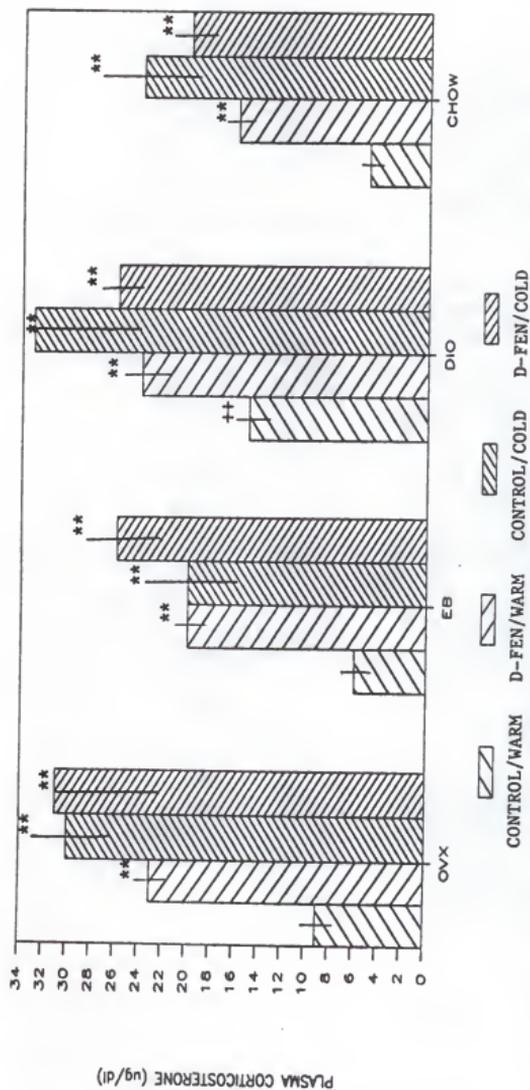


Figure 35. Effects of d-fenfluramine treatment on basal nighttime corticosterone concentrations and on the stress-induced rise in plasma corticosterone. Plasma corticosterone concentrations were measured under basal nighttime conditions (25 °C; WARM) on the third day of d-fenfluramine administration and after 4 hours of cold exposure (4 °C; COLD) on the fourth day of d-fenfluramine administration. Plasma corticosterone concentrations are expressed in micrograms corticosterone/deciliter plasma for d-fenfluramine-treated rats (D-FEN) and untreated controls in the four dietary or surgical condition groups.

** Different from corresponding group, untreated control at 25 °C, $p < .01$, Newman-Kuel's post hoc t-test.

++ Different from CHOW untreated control group at 25 °C, $p < .01$.

regimen used in this experiment was not effective in reducing the elevated triglyceride concentrations. Previous reports on the ability of fenfluramine to reduce the stress-induced rise in triglycerides found this effect only at high drug doses (Brindley, 1985). In high concentrations, d-fenfluramine decreases circulating triglycerides (Brindley, 1983; Brindley et al., 1985). In those experiments, rats were maintained on a high fat diet and the stressor was a fructose load. It is possible that the type of stress is important: thus, d-fenfluramine may reduce the triglyceride rise in response to a metabolic stress, but have no effect on the triglyceride peak in response to other types of stress such as the cold exposure used in the present experiment.

The effect of high doses of d-fenfluramine on the stress-induced peak in plasma triglyceride concentrations may be achieved by a combination of mechanisms including (a) direct inhibition of phosphatidate phosphohydrolase (Brindley, 1983); (b) a long-term decrease in stress-induced release of corticosterone and adrenal catecholamines that would decrease the total levels of enzyme and (c) increased insulin sensitivity that would oppose the glucocorticoid-induced increase in triglyceride synthesis and release from liver (Brindley et al., 1985).

The effects of d-fenfluramine treatment on plasma corticosterone concentrations in the present experiment are consistent with the results of Experiments 1 and 2. Plasma corticosterone

concentrations were elevated in dietary obese rats compared to untreated control rats in the other dietary or surgical condition groups. Plasma corticosterone concentrations were elevated by cold exposure in all dietary or surgical condition groups. Fenfluramine treatment elevated corticosterone levels in all condition groups at 25 °C, but did not affect the high corticosterone concentrations at 4 °C. The elevation in plasma corticosterone concentrations induced by d-fenfluramine treatment was comparable to the corticosterone peak induced by 4 hours of cold exposure and both were at or near peak levels.

Previously reported effects of chronically administered high dosages (25 mg/kg/day) d-fenfluramine are different from acute effects and different from the effects of the chronic treatment regimen used in the present experiments. In previously reported experiments, basal corticosterone levels were normal and the stress-evoked rise in plasma corticosterone, catecholamines and FFA were substantially decreased (Brindley et al., 1985). Those effects were seen with a high drug dosage (25 mg/kg/day), but not with a lower a dosage (2.5 mg/kg twice daily).

The d-fenfluramine-induced increase in plasma corticosterone concentrations found in the present experiments is similar to the acute affect of higher dosages. This is consistent with a 5-HT mechanism for the d-fenfluramine-stimulated rise in glucocorticoids: in a paradigm in which 5-HT is not depleted,

glucocorticoid levels remain elevated. Acutely, d,l-fenfluramine stimulates corticosterone release (Fuller et al., 1981; McElroy et al., 1984; Schettini et al., 1979) and has mild sympathomimetic actions including increased plasma NE (Calderini et al., 1975; Lake et al., 1979). These effects may be mediated in part by central or peripheral 5-HT: exogenously administered 5-HT stimulates the sympathetic nervous system and stimulates release of corticotrophic releasing hormone.

Experiment 4: Effects of d- and d,l-Fenfluramine
on Lateral Hypothalamic Self-Stimulation

The importance of central actions of fenfluramine on appetite suppression and weight loss have been discussed. The effects of fenfluramine on brain serotonin (5-HT), dopamine (DA) and brain opioids have also been discussed. Brain DA turnover is stimulated (Jori and Bernardi, 1972) and apparent tolerance does not develop to this effect (Jori and Dolphini, 1977). The l-isomer is more potent than the d-isomer in this regard (Jori and Dolphini, 1974). The effects of fenfluramine on brain opioids appear to be related to 5-HT activity although the nature of this relationship is not clear. Both brain DA and brain opioids have been implicated in stress-induced eating and as possible mediators of the rewarding properties of food.

Electical current delivered via electrodes implanted in the area of the medial forebrain bundel of the lateral hypothalamus will produce both consumatory behaviors and reinforcing effects.

Animals readily learn to press a bar in order to deliver intracranial stimulation (ICSS). When food is present, electrical stimulation to this area will induce consumatory behaviors including eating and gnawing.

The effects of injections of different doses d- and d,l-fenfluramine on the responding of rats trained to press a bar to self-deliver electrical current to the lateral hypothalamus via implanted stainless steel electrodes were assessed. Most rats readily learned to press a bar to receive intracranial self-stimulation (ICSS) by simple behavioral shaping procedures. Rats were trained to bar press at the lowest stimulation level that produced essentially constant responding. Daily training and testing sessions were 1 hour/day in the later part of the light cycle (15:00-17:00 hours). When the rats were responding reliably, baseline measurements were taken. The number of responses was recorded at the end of each 15 minute interval.

Following 3 days of baseline measurements, drug treatment began. Rats were tested with increasing dosage of d- and d,l-fenfluramine. Each dosage was administered for a 3-day period with a drug-free interval of at least 1 day between the different dosages and a 3-day drug-free period at the end of the experiment. Drug dosages were 2, 4 and 8 mg/kg of both compounds. Each rat received both drugs; half received the series of d-fenfluramine first and half received the racemate first.

When ICSS testing was completed, the rats were screened for "stimulation bound" eating or gnawing by placing them in a stimulation chamber with food, but no bar, present. Electrical stimulation matched to a level at which the rats had responded for ICSS, was delivered at a rate of 5 pulses/second for 30 seconds intervals (30 seconds on/30 seconds off). If the rats did not respond to this electrical stimulation of the brain (ESB), the voltage was gradually increased to see if a response could be evoked. Rats were judged positive or negative for the response.

Following the termination of the study, the rats were given an overdose of pentobarbital anesthesia and perfused with 10% formalin solution. The brains were removed and stored in formalin solution, and later cut on a freezing microtome at a thickness of 30 μ m and stained with methylene blue. Electrode placement was verified by microscopic examination of the brain sections.

The baseline rates of ICSS responding ranged from 1108-7432 responses/hour. Patterns of ICSS responding of two representative rats are shown in Figures 36 and 37. The rat represented in Figure 36 received d,l-fenfluramine first and the rat represented in Figure 37 received d-fenfluramine first. In both cases, the racemate was more effective in suppressing the rate of responding. Fifteen of the total 20 rats were judged positive for ESB-induced eating; however, there was no discernable difference in rates of responding or in the drug effects in animals that ate in response to stimulation compared to those that did not.

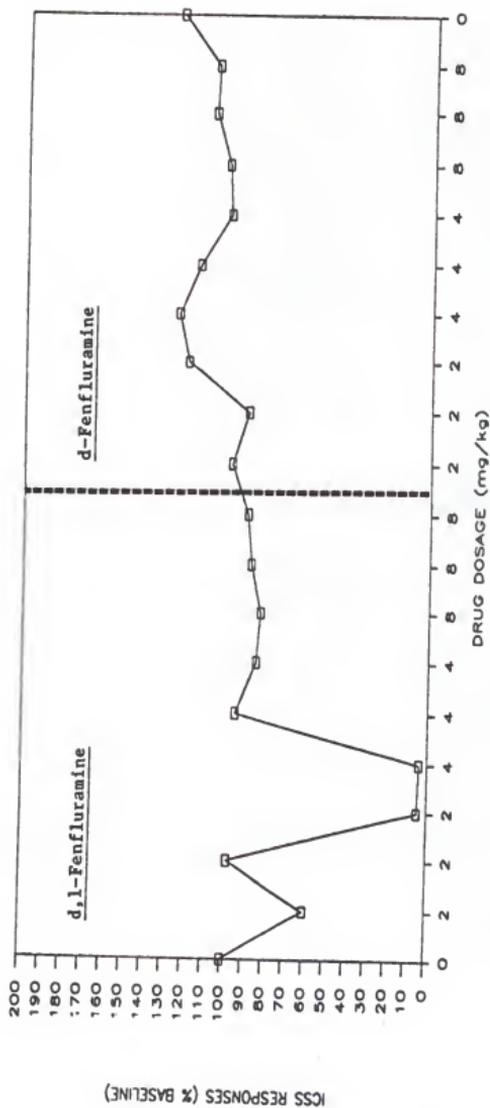


Figure 36. Effects of d- and d,l-fenfluramine on intracranial self-stimulation (ICSS). Shown are total daily operant responses of a rat trained to press a bar in order to receive ICSS in the area of the lateral hypothalamus. The response rates (bar presses/hour) are expressed as percentages of the baseline response rate for a series of drug dosages (2-8 mg/kg d- and d,l-fenfluramine). Each drug dosage was administered for 3 days consecutively, with a drug-free day between each series of drug dosages. The rat represented in this figure received the series of d,l-fenfluramine dosages first, followed by the series of d-fenfluramine dosages.

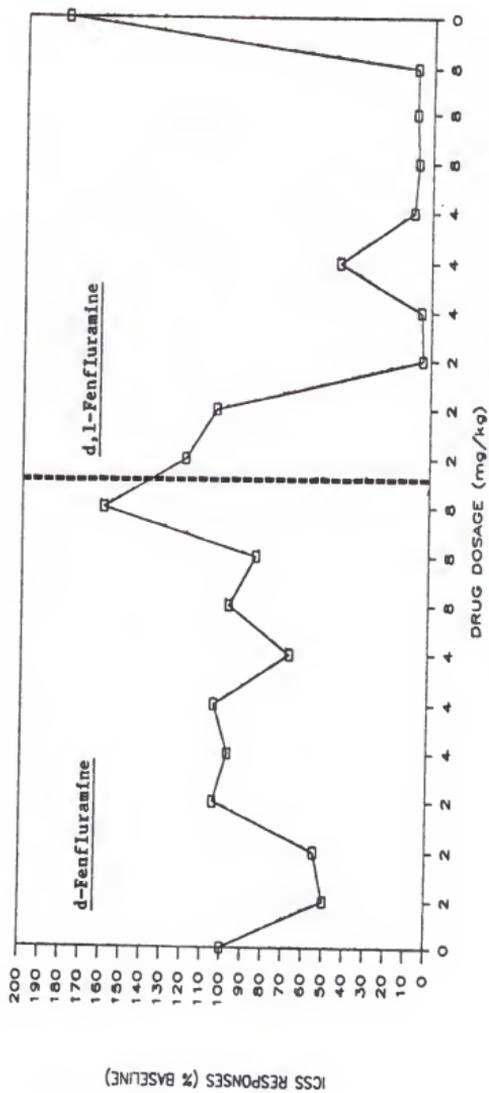


Figure 37. Effects of d- and d,l-fenfluramine on intracranial self-stimulation (ICSS). Shown are total daily operant responses of a rat trained to press a bar in order to receive ICSS in the area of the lateral hypothalamus. The response rates (bar presses/hour) are expressed as percentages of the baseline response rate for a series of drug dosages (2-8 mg/kg d- and d,l-fenfluramine). Each drug dosage was administered for 3 days consecutively, with a drug-free day between each series of drug dosages. The rat represented in this figure received the series of d-fenfluramine dosages first, followed by the series of d,l-fenfluramine dosages.

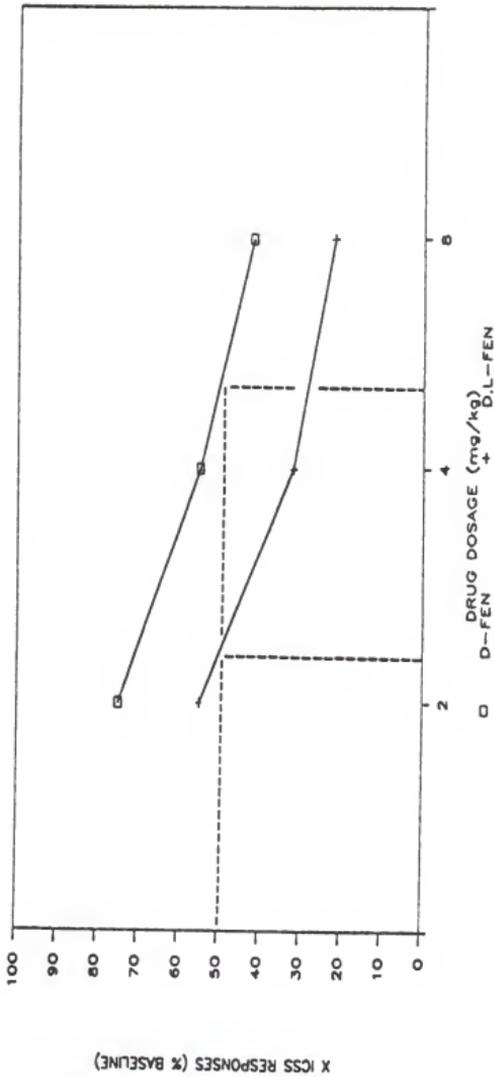


Figure 38. Dose response curves for the effects of d- and d,l-fenfluramine on intracranial self-stimulation (ICSS). Shown are mean response rates (bar presses/hour) expressed as percentages of baseline response rates for rats treated with a series of increasing dosages (2, 4 or 8 mg/kg) d- or d,l-fenfluramine. Also shown are the extrapolated ED_{50} estimates of the dosage of each drug at which the mean response rate is 50% of the mean baseline response rate.

Dose-response curves were computed by converting the number of responses per hour of each rat on the first day of administration of a given drug dosage to a percentage of that rat's baseline responses. The dose-response curves shown in Figure 38 represent the average percentage of baseline responses for all rats tested. The ED₅₀ doses were 5.5 mg/kg d-fenfluramine and 2.3 mg/kg d,l-fenfluramine.

Experiment 4: Discussion

Both d- and d,l-fenfluramine were effective in suppressing ICSS; however, the racemate was more potent in this regard. This implies that a brain DA mechanism is involved. Hoebel et al. (1986) have reported that d-fenfluramine decreases ICSS responding in rats with electrodes in the area of the lateral hypothalamus that was also associated with ESB-induced eating and excitation of taste neurons. Those authors concluded that d-fenfluramine inhibits feeding reward and taste reward and that these effects are mediated by release of 5-HT in the lateral hypothalamus.

The results of the present experiment do not support that hypothesis, but rather suggest that a dopaminergic mechanism is primarily involved in the suppression of ICSS responding by d- and d,l-fenfluramine. Both d- and l-fenfluramine stimulate brain DA turnover (Jori and Dolphini, 1974; Garattini et al., 1975). Although the l-isomer is more potent in this regard, d-fenfluramine is at least as potent as an equal dose of amphetamine

(Rowland and Carlton, 1986). This does not rule out the possibility that brain 5-HT or opioids are also involved in this effect of fenfluramine. It does imply that brain 5-HT is not the primary mediator since the d-fenfluramine is more effective in stimulating brain 5-HT activity but less effective in suppressing ICSS than equivalent doses of d,l-fenfluramine.

Both central and peripheral opioids may be involved in feeding behavior and energy regulation (Majeed et al., 1986; reviewed in Morley et al., 1983). There is also evidence for interactions between brain opioid and monoamine systems the exact nature of which is unclear and may be reciprocal (Dellavedova et al., 1982). Exogenous administration of opioids increases 5-HT synthesis and release (Smialowska and Bal, 1984) and changes in hypothalamic and striatal 5-HT activity are associated with changes in opioid levels in these areas (Groppetti et al., 1984; Harsing et al., 1982). Depletion of brain 5-HT has been reported to increase brain opioid levels (Han et al., 1980). This probably reflects neuropeptide accumulation due to decreased utilization rather than increased synthesis (Mocchetti et al., 1985).

There is evidence that brain opioid and 5-HT system interactions may be influenced by fenfluramine and in turn, affect anorexia and/or tolerance. Dextrofenfluramine (15 mg/kg/day x 5 days) has been associated with increased hypothalamic concentrations of met-enkephalin and beta-endorphin that last up to 10 days

(Harsing et al., 1982). Those authors suggested that the anorectic effects of d-fenfluramine may be mediated by decreased opioid activity. Others (Groppetti et al., 1984) have proposed that the fenfluramine-induced release of brain 5-HT is modulated by the opioids and that anorexia is dependent upon 5-HT release. Following depletion of 5-HT with a single large dose or repeated small doses of d,l-fenfluramine, acutely anorectic doses of the drug were not effective in further depleting 5-HT and did not produce anorexia. Increased hypothalamic and striatal met-5-enkephalin were also associated with decreased 5-HT in that study. Depletion of 5-HT was potentiated by pretreatment with morphine and antagonized by naloxone. In d,l-fenfluramine-tolerant rats, morphine pretreatment restored the ability of fenfluramine to further deplete 5-HT and to produce anorexia (Groppetti et al., 1984).

Theories concerning ICSS behavior include drive-reduction, incentive-motivation and reward. In accordance with drive reduction theories, when a stimulus elicits a consumatory response, that response ordinarily persists until the stimulus is removed and satiation occurs. In a hungry animal, exteroceptive and interoceptive food stimuli are rewarding. As the hunger is diminished, the same stimuli become less rewarding or aversive. Thus ICSS can be described as a consumatory behavior that does not satiate. The reinforcing effects of ICSS have also been attributed to an

appetitive-motivational system. Stimulation produces a central state that induces the animal to obtain reinforcement by appropriate motor responses. Olds (1976) conceptualized the central nervous system as a reward and learning machine with motivation, reward and learning the key words in the programming of behaviors. Subjects learn to do things if they are rewarded for doing so and if they are motivated. Behavior is steered and eventually terminated by rewards.

Two approaches have been used to study the interrelationship of ESB and motivation. One is to vary motivation (e.g. starvation or overfeeding) and look at ICSS behavior and the other is to deliver ESB and examine the effects on motivated behaviors.

Responding rates for ICSS are greatest when stimulation electrodes are located in the lateral hypothalamus, but maps of "reward" sites extend from the olfactory paleocortical area of the telencephalon spreading across the floor of the forebrain and midbrain and along a path near the roof of the medulla.

Rates of ICSS follow a circadian rhythm with the highest rates occurring at night and peaking in the final hours of the dark cycle. The ICSS rhythm matches rhythms in food and water intake and in body temperature. When sweetened milk is provided as food, the peak ingestion is earlier and the variability in the pattern is increased (Terman and Terman, 1976).

In the lateral hypothalamus, ICSS responding is rapid and animals show signs of marked sympathetic nervous system arousal including tachycardia, hyperthermia, hypertension and general stress responses including elevation in plasma glucocorticoids and catecholamines.

Electrical stimulation delivered to sites that support ICSS elicits consumatory behaviors, the nature of which is dependent, upon the location of the electrode as well as the availability of goal objects. Eating and drinking in response to ESB are not like those behaviors in hungry or thirsty animals, but appear stereotypical. Rather than eliciting natural-like motivated behaviors, ESB may evoke well-practiced response patterns. Thus ESB at certain sites may evoke or sensitize a motor response that tends to channel behavior in a given direction. These are fragmentary responses that do not duplicate natural motivational states and may be programmed in the brain stem (Valenstein, 1976).

Rats made obese by ESB-induced eating decrease their ICSS response rate as a function of weight gain. An animal that increases food intake or ICSS may be responding to either increased positive feedback (appetite) or decreased negative feedback from satiety stimuli. Rolls (1976) located a group of neurons in the lateral hypothalamus that increased their firing rate in hungry animals or in response to naloxone injection in morphine addicted animals and could be "turned off" by food,

morphine injection or ESB. Furthermore, single units in the lateral hypothalamus that are activated (either excited or inhibited) by ESB are also activated by natural rewards such as the sight and taste of food but not by eating itself. Thus hypothalamic activation that can motivate an animal to eat will motivate other behaviors as well (Rolls, 1976).

The majority of evidence points toward the involvement of brain catecholamines, especially DA, as mediator of the rewarding effects of ICSS. Amphetamine stimulates ICSS responding and this effect seems to be related to its activity as a DA agonist. Accordingly ICSS responding is increased by dopamine-beta-hydroxylase inhibitors and is decreased by DA, but not NE, antagonists (Wauquier, 1976). Amphetamine also stimulates other operant responses, implying that general motor arousal may be responsible; however, the rewarding properties of amphetamine are clearly indicated. Animal will self-administer amphetamine and in humans, it produces euphoria and has a high abuse liability. In a review of pharmacological and anatomical evidence, Wise (1976) concluded that 5-HT is not critical to the rewarding effects of ICSS.

In humans, ESB in the area of the medial forebrain bundle produces sensations of general euphoria, positive mood change, increased talking, pleasurable physical sensations, relief of anxiety and pain reduction. A single session may relieve chronic

pain for up to a week (Delgado, 1976). While there is some relationship between electrode placement and the type of sensation reported, this is not consistent either between subjects or in the same subject on different days. Situational factors also have a strong influence.

There are similarities between ESB-induced eating and tail pinch-induced eating. Tail pinch elicits similar behaviors and is thought to be dependent on brain DA (Antelman and Caggiula, 1979). The nigrostriatal DA system is important in sensory and motor function and facilitation of DA increases responsivity to external and internal stimuli (Wise, 1976).

In the laboratory and in nature, there are many examples of stress-induced eating associated with fighting and sexual behavior (Morley et al., 1983; Antelman and Caggiula, 1979) or other similarly activating conditions. Furthermore, stressful conditions that elicit eating in certain environmental conditions may elicit sexual or aggressive behavior under other circumstances (Morley et al., 1983). Tail pinch-induced eating has been proposed as an animal model of stress-induced eating (Antelman and Caggiula, 1979; Morley et al, 1983). In rats, mild tail pinch reliably produces behaviors similar to naturally occurring stress-related behaviors. Eating, licking and gnawing are especially prevalent. Tail pinch behaviors may be affected by brain 5-HT and brain opioids. Tail pinch behaviors are attenuated by

pharmacological manipulations that enhance 5-HT activity in the brain (Antelman and Caggiula, 1979). The opiate antagonist naloxone suppresses tail pinch-induced eating (Morley et al., 1983) whereas the synthetic opioid d-ala-met-enkephalinamide (DAME) stimulates eating (Morley et al., 1983). Acutely, d,l-fenfluramine abolishes tail pinch-induced eating, although tolerance develops to this effect (Antelman and Caggiula, 1979). In contrast, amphetamine anorexia is reversed by tail pinch (Antelman et al., 1979).

Reports of increases in met-5-enkephalin associated with chronic administration of d- or d,l-fenfluramine suggest that this brain opioid may be involved in d,l-fenfluramine anorexia (Groppetti et al., 1984; Dellavedova et al., 1982; Harsing et al., 1982) and tolerance (Groppetti et al., 1984).

Peripheral administration of morphine or icv. injection of met-5-enkephalin or the synthetic analog DAME enhance DA turnover in rat striatum. Morphine and, to a greater extent, DAME stimulate DA release in vivo (Chesselet et al., 1981).

Exogenously administered opiates (e.g. morphine) can stimulate appetite and also ICSS responding. The opiate antagonist naloxone inhibits food intake and decreases ICSS responding. Depletion of 5-HT may stimulate food intake under some conditions and also inhibit ICSS responding. With administration of d,l-fenfluramine, levels of brain opioids gradually increase

suggesting that release is inhibited. Opioids may increase the synthesis of 5-HT and administration of morphine releases 5-HT in previously 5-HT-depleted animals.

CHAPTER V

CONCLUSIONS

Fenfluramine is an appetite suppressant drug used in the treatment of obesity. Until recently, only the racemate was available clinically and was used in most experimental research. The two enantiomers have different properties however, and d-fenfluramine is the more potent anorectic. Dextrofenfluramine (Isomeride) is currently available in Europe and is being tested for marketing in the U.S.

The relevance of animal studies to clinical applications in eating and weight disorders has been seriously questioned in recent years. Experimental results, in many cases, do seem to be consistent with clinical observations. There are two important ways in which procedures for animal research on the pharmacological actions of fenfluramine often differ from the way the drug is used clinically.

(1) Clinically fenfluramine is administered in slow-release capsules or tablets taken twice daily. The dosage is gradually increased during the first weeks of treatment to approximately 1-2 mg/kg/day d,l- and 0.5-1 mg/kg/day d-fenfluramine. There is a gradual accumulation of drug in adipose and other tissues until steady-state plasma levels are reached (Campbell et al., 1979). In rats, fenfluramine is typically administered in single daily

injections, often at high dosages. The half-life of fenfluramine is approximately 24 hours in humans and 4 hours in rats. The half-life of the active metabolite, norfenfluramine, is approximately twice that of fenfluramine in both humans and rats. Drug metabolism in humans is such that the concentration of the parent compound is generally greater than that of the metabolite. In rats however, plasma concentrations of the metabolite are relatively greater within 2-6 hours of administration (Caccia et al., 1982). Norfenfluramine, unlike its ethylated parent, appears to have direct actions at 5-HT postsynaptic receptors (Borsini et al., 1982; Mennini et al., 1985). When d,l-fenfluramine is administered, the l-isomer is preferentially metabolized in humans (Beckett and Brookes, 1970) whereas the d-isomer is metabolized faster in rats (Morgan et al., 1972). Thus the more active isomer is more quickly eliminated in rats, but not in humans.

Much of the information on both the central and peripheral actions of fenfluramine has been obtained from in vitro preparations using high drug concentrations. Studies conducted in vivo have typically used one- or two-daily injections of a single drug dosage that is often well above the ED_{50} for producing anorexia in the species being tested. In previous experiments conducted in our laboratory, it was noted that rats treated with d,l-fenfluramine, at a dosage of 10 mg/kg or greater, behave in a manner that is strikingly different from rats given lower dosages. In

reviewing the literature, one finds that the vast majority of studies on the biochemical actions of both d- and d,l-fenfluramine report using relatively high drug dosages (≥ 7.5 mg/kg d- or ≥ 10 mg/kg d,l-fenfluramine). In the cases where a range of drug dosages is tested, effects at high dosages often are not found at the lower dosages.

It was hypothesized that procedures using intermittent administration of a constant, high drug dose may produce effects that are not strictly applicable to the clinical situation and that this may be a reason for some of the discrepancies between clinical and experimental observations. In order to model more closely a clinical treatment regimen, d-fenfluramine was administered continuously via chronically indwelling osmotic pumps that delivered 3 mg/kg/day at a rate of 0.5 ul/hour. Considering the difference in the plasma half-life of fenfluramine in humans and rats, this dosage is comparable to the customary clinical dosage of 0.5 mg/kg/day.

(2) Clinically fenfluramine is administered to obese patients, usually females. The animal models most commonly used in the study of ingestive behavior involve structured feeding schedules or food deprivation paradigms in normal weight laboratory rats (often males) fed a uniform, bland diet. It is not surprising that the results from these experiments do not closely match the eating patterns of obese humans. In the studies

reported here, animal models of obesity that incorporate factors relevant to human obesity were selected and range of behavioral and both central and peripheral biochemical variables, and their interactions were examined in order to develop an experimental situation that would be objectively and conceptually more similar to that seen clinically in humans.

Two animal models of obesity were used in the present experiments: diet-induced obesity produced by feeding a varied, palatable diet (DIO) and obesity developed subsequent to ovariectomy (OVX). The model of diet-induced obesity seems to be relevant to many of cases of human obesity. Palatability and food composition are extremely important determinants of food intake and availability of a variety of highly palatable foods apparently can override early satiety signals. This model also may be relevant in some regards to special cases of overeating such as *Bulimia Nervosa*, which is characterized by ingestion of large amounts of palatable (high carbohydrate, high fat) foods.

Ovariectomy rapidly and reliably produces weight gain and obesity in rats that can be reversed by administration of physiological levels of estrogen. Females of many species, including humans and rats, have a higher percentage of body fat, the degree and disposition of which is affected by ovarian hormones (Wade and Gray, 1979). Females also have a greater ability to conserve energy stores and alter food efficiency. Naturally occurring or

experimentally induced fluctuations in the levels of circulating gonadal hormones produce changes in body weight and fat content, as well as changes in relevant behaviors such as food intake and voluntary exercise (Wade and Gray, 1979).

Features common to these and other animal obesity models and possible causes of obesity that were examined include (a) energy imbalance due to increased food intake, decreased metabolic rate, decreased thermogenesis or increased efficiency of metabolism; (b) increased adiposity due to hyperplasia and/or hypertrophy of adipose tissue; (c) hyperinsulinemia and insulin resistance; (d) enhanced lipid accumulation due to increased lipogenesis and/or decreased lipolysis and (e) hyperlipidemia.

Effects of Chronic d-Fenfluramine Treatment
on Food Intake and Body Weight

Feeding of a varied, palatable diet sustained increases in food intakes and body weights of female Sprague-Dawley rats. The mean baseline daily caloric intake of rats that were fed a modified cafeteria diet consisting of Chow, cookies and sweetened milk was 53% higher than that of rats maintained on Purina Chow pellets alone. Thus diet-induced obesity was maintained by high caloric intake; however, this was not the case for ovariectomy-induced obesity. The baseline caloric intake of Chow-fed ovariectomized rats was not significantly different from that of unoperated Chow-fed rats. After 10 weeks on the modified cafeteria diet, rats had body weights that were 25% higher than unoperated Chow-fed rats.

During the 10-week pretreatment period, ovariectomized Chow-fed rats gained almost exactly the same amount of weight as dietary obese rats.

In Experiment 1, the effects of 4 weeks of continuous administration of 3 mg d-fenfluramine/kg/day via osmotic minipumps were assessed in female Sprague-Dawley rats from the four dietary or surgical condition groups: obese ovariectomized (OVX); ovariectomized with estradiol benzoate replacement (EB); dietary obese (DIO) and unoperated Chow-fed (CHOW). The effects of this treatment regimen on food intake, body weight and food efficiency were compared to untreated control rats in each the four dietary or surgical condition groups during the 4-week treatment period and during a 2-week drug-free posttreatment period.

On the first 2 days of administration, d-fenfluramine treatment suppressed food intake of ovariectomized and dietary obese rats and unoperated Chow-fed rats by a similar degree (52-63%); however, d-fenfluramine had no significant effect on the food intake of estradiol-treated rats, even on the first 2 days of administration.

By days 7-8 of the treatment period, mean food intakes had returned to baseline levels in all d-fenfluramine-treated rats except the dietary obese rats. The mean daily caloric intake of dietary obese rats that received d-fenfluramine treatment remained at approximately 75% of baseline intake on days 7-8 and 13-14 of

chronic drug treatment, but had returned to baseline levels by days 27-28. There were no significant effects of previous d-fenfluramine treatment on food intakes of rats in any of the four dietary or surgical condition groups during the posttreatment period.

The efficacy of d-fenfluramine in reducing food intake in the different dietary or surgical condition groups was more closely related to baseline food intake than to pretreatment body weight: the food intake of d-fenfluramine-treated ovariectomized rats was not affected to a greater extent than that of unoperated Chow-fed rats, although their body weights at the beginning of the d-fenfluramine treatment period were comparable to body weights of dietary obese rats. The baseline food intake of dietary obese rats was 53% higher than Chow-fed rats; therefore, the 25% reduction in food intake of these rats that was associated with d-fenfluramine administration was not sufficient to decrease their mean daily caloric intake to the level of Chow-fed rats.

During the 2-week posttreatment period, food intakes of rats that had received d-fenfluramine were compared to food intakes of untreated controls from each of the four dietary or surgical conditions. There were no significant effects of previous d-fenfluramine treatment on posttreatment food intake.

In order to determine if d-fenfluramine treatment differentially affected consumption of the different dietary components in

the cafeteria diet, the percentage of total caloric intake contributed by each component was measured. The effect of d-fenfluramine on the food intake of dietary obese rats was to decrease total caloric intake by reducing intake of all of the dietary components, but by different degrees. The percentage of total calories consumed as Chow was decreased while the percentage consumed in milk was increased. The most likely explanation for this involves palatability of the different foods. When food intake is decreased by d-fenfluramine, the bland Chow is affected most strongly while the sweeter, more palatable foods are spared.

The effects of d-fenfluramine on the different dietary components argues against the hypothesis that d-fenfluramine works via a brain 5-HT mechanism to selectively suppress carbohydrate intake while sparing protein intake (e.g. Wurtman and Wurtman, 1984). In the present experiment, the sweetened lowfat milk was 20% protein whereas the Chow and cookies were 23% and 5% protein respectively. The carbohydrate content of the sweetened milk was 80%; the cookie, 70% and the Chow, 50%. Clearly in this experiment, carbohydrate intake was not suppressed to a greater extent than protein intake.

Continuous administration of d-fenfluramine for 4 weeks resulted in a weight loss in ovariectomized rats, significantly reduced weight gain in dietary obese and unoperated Chow-fed rats

and had no significant effect on the body weights of estradiol-treated rats. Fenfluramine was clearly more effective in reducing the body weights of ovariectomized rats than in dietary obese and unoperated Chow-fed rats whereas it was totally ineffective in reducing body weights of estradiol-treated rats. It is not clear why d-fenfluramine was more effective in reducing the body weights of obese ovariectomized rats than dietary obese rats. The d-fenfluramine-induced reduction in food intake was prolonged in dietary obese rats compared to other groups including ovariectomized rats; however, as noted above, the effect was not sufficient to reduce the daily caloric intake of dietary obese rats to a level comparable to that of Chow-fed rats in the OVX, EB and CHOW dietary or surgical condition groups. This implies that the availability of a palatable diet may override mechanisms that reduce food intake and body weight. It is possible that the ovariectomized rats are particularly sensitive to some metabolic actions of d-fenfluramine. If indeed fenfluramine is more effective in females the reasons may be related; thus, the drug's effectiveness may be influenced by hormonal status.

During the 2-week posttreatment period, dietary obese, estradiol-treated and unoperated Chow-fed rats that had received d-fenfluramine gained comparable amounts of body weight. Within the DIO condition group, the weight gain of rats that had received d-fenfluramine was 50% greater than that of untreated control rats

in that condition group. Within the CHOW condition group, rats that had received d-fenfluramine gained 78% more weight than untreated controls. Thus rats in those condition groups that had received d-fenfluramine treatment gained substantially more weight than controls during the posttreatment period although their food intakes were not significantly greater; therefore, increased weight gain during the posttreatment period occurred in the absence of significantly increased food intake. During the post-treatment period, ovariectomized rats that had been treated with d-fenfluramine continued to lose weight although at a much slower rate than during drug treatment and the total 2-week weight change was not significantly different from untreated ovariectomized rats that showed only a small weight gain during the 2-week posttreatment period.

The lack of effect of d-fenfluramine administration on food intake and body weight of ovariectomized rats receiving estradiol replacement and the increased efficacy in ovariectomized rats not receiving estradiol are consistent with a set-point model. Accordingly estradiol-treated rats are maintaining lowered body weights relative to ovariectomized or unoperated rats; therefore, subsequent treatment with d-fenfluramine does not further decrease body weight. Conversely ovariectomized rats that do not receive estradiol are maintaining their body weights at an elevated level and hence, are more sensitive to the drug's effects. The increased

weight gain during the posttreatment period seen in dietary obese, estradiol-treated and unoperated Chow-fed rats is also consistent with a set-point model according to which d-fenfluramine acts to lower body weight set-point. Similar arguments are among the most common in set-point theory. This provides an easy explanation for the increased efficacy of d-fenfluramine in obese animals and for the enhanced weight gain following the termination of treatment, but contributes very little to the understanding of body weight regulation or of the actions of drugs affecting appetite and body weight.

Although the effects of d-fenfluramine in obese rats do seem to fit a set-point model, this is not a complete explanation and it does not encompass all the data. Such a model is of little heuristic value in explaining the effects of d-fenfluramine or other anorectic drugs on food intake and body weight. Rather than using hypothetical constructs to explain physiological phenomena, a much more productive approach is to examine possible mechanisms by which this can occur.

Food efficiency [total weight gain (grams)/total kilocalories food ingested] was considerably higher in ovariectomized estradiol-treated rats than in the other dietary or surgical condition groups. During the period of time for which food efficiency was measured, estradiol-treated rats were gaining weight at a faster rate than obese ovariectomized or unoperated Chow-fed rats and at

a rate comparable to dietary obese rats with their high caloric intake. This suggests that after a period of time, the weight-reducing effects of estradiol treatment are overridden by mechanisms to conserve body weight so that continued weight loss does not occur. During this period, food efficiency was very low in ovariectomized rats that did not receive estradiol replacement. This was associated with a decreased rate of weight gain in these rats. If food efficiency had been measured in the early phases following ovariectomy when weight gain was most rapid, it might have been considerably higher in those rats that did not receive estradiol replacement. Administration of d-fenfluramine had no significant effect on the relative mean food efficiency of dietary obese, estradiol-treated or unoperated Chow-fed rats.

During the 2-week posttreatment period, the estimated food efficiency of untreated control rats in each of the four dietary or surgical condition groups was similar to their food efficiency during the preceding 4-week period. Control rats in the EB group had higher [weight gain/kilocalorie ingested] ratios than did untreated control rats in the other condition groups. There was no significant effect of previous d-fenfluramine treatment on food efficiency.

The failure to find a significant effect of d-fenfluramine on food efficiency is curious. During d-fenfluramine treatment, unoperated Chow-fed rats had lowered weight gain, but not

significantly lowered food intake. During the posttreatment period, the rate of weight gain of rats in both the DIO and CHOW condition groups was increased relative to untreated control rats in those groups, but again, food intake was not significantly affected. Thus food efficiency estimated from [weight gain/kilocalorie ingested] may not be a reliable index in these situations.

The finding that d-fenfluramine is more effective in obese than in lean rats implies that data obtained from normal weight laboratory animals may not be directly applicable to the understanding of d-fenfluramine's effects in obese humans.

In Experiment 2, the effects of 4 weeks of administration of 3 mg d-fenfluramine/kg/day were compared to the effects of 4 weeks of dietary restriction in obese ovariectomized (OVX), dietary obese (DIO) and unoperated Chow-fed (CHOW) rats. Fenfluramine was administered continuously via osmotic minipump throughout the 4-week treatment period. The rats in the dietary restriction groups in each of OVX, DIO and CHOW dietary or surgical conditions received no drug treatment, but were given access to only 75% of their baseline daily food intakes during the 4-week treatment period. The purpose of this experiment was to compare the effects of d-fenfluramine administration to a 25% dietary restriction in the absence of drug treatment.

The decision to use a 25% dietary restriction was based on a preliminary experiment in which the mean food intake of dietary

obese rats was reduced to approximately 75% of baseline by this d-fenfluramine treatment regimen. In the present experiment, the mean 2-day caloric intakes of dietary obese rats analyzed at specific days during the treatment period was approximately 75% of baseline through the second week of treatment; however, the 28-day mean caloric intake in the present study was 86% of baseline and in d-fenfluramine-treated rats from the other two condition groups, food intakes were not significantly different from untreated control values after the first week of d-fenfluramine administration.

Measurements of food intake, body weight and food efficiency in rats from the DIO and CHOW condition groups were continued for a 2-week drug-free posttreatment period during which diet-restricted rats were returned to ad libitum feeding conditions. The rats in the d-fenfluramine-treated and untreated control groups were the same as in Experiment 1. The purpose of this experiment was to compare the posttreatment effects of d-fenfluramine and dietary restriction in lean and obese rats.

During the 2-week posttreatment period, food intakes of both Chow-fed and dietary obese rats that had been on a restricted diet or had received d-fenfluramine were not significantly different from the food intakes of rats that had received no treatment.

Administration of d-fenfluramine for 4 weeks resulted in a weight loss in ovariectomized rats and significantly reduced

weight gain in dietary obese and Chow-fed unoperated rats. This treatment regimen was equally effective as 25% caloric restriction in dietary obese rats, but was less effective in reducing body weights of obese ovariectomized and unoperated Chow-fed rats. Dietary restriction produced weight loss in the ovariectomized and unoperated Chow-fed rats and significantly reduced weight gain in dietary obese rats compared to untreated control rats in the corresponding groups. This is not surprising since d-fenfluramine failed reduce food intakes by 25% throughout the experiment. Because of this, it is not possible to make definitive conclusions on proposed antiobesity actions of d-fenfluramine that may occur independently of decreased food intake and body weight.

The difference in the effectiveness of the two procedures was most pronounced in ovariectomized rats. The body weights of obese ovariectomized rats were decreased both by dietary restriction procedures and by d-fenfluramine treatment to a greater extent than rats in the other dietary or surgical condition groups. The excessive weight loss in ovariectomized rats on a restricted diet was not expected. Ovariectomized rats have been reported to gain weight when pair-fed to ovariectomized estradiol-treated rats and the obesity that develops following ovariectomy is not dependent upon hyperphagia (Roy and Wade, 1977). The time interval following ovariectomy may be an important factor as discussed in Experiment 1. Ovariectomized rats show an initial dynamic phase

of weight gain that lasts about 1 month and then levels off to a trajectory similar to control rats (Wade and Gray, 1979). Thus during the later "plateau" phase of obesity, ovariectomized rats may be more sensitive to weight reduction procedures.

During the posttreatment interval, rats that had received d-fenfluramine or dietary restriction gained weight more rapidly than their controls even though their daily mean food intake was not significantly increased relative to controls. The increased weight gain of previously restricted rats was accompanied by a marked (94-123%) increase in food efficiency. This dramatic increase in food efficiency in rats that were recovering from dietary restriction is consistent with previously reported increases in food efficiency and weight gain following episodes of experimentally imposed weight loss (Coscina and Dixon, 1983; Pertschuk et al., 1983; Rolls et al., 1980).

Effects of d-Fenfluramine on Adipocyte Size

A decrease in lipid storage associated with weight loss may be reflected in a decrease in adipocyte size in one or more fat depots. To determine if weight loss associated with chronic d-fenfluramine treatment is a result of decreased lipid storage, the diameters of white and brown adipocytes were measured.

The mean diameters of inguinal white adipocytes were similar in untreated control rats from the OVX (43 μ m), DIO (44 μ m) and CHOW groups (43 μ m), but the mean adipocyte diameter of rats from

the EB group, was 22% smaller than that of the OVX group. This indicates that the increased adiposity of obese ovariectomized and dietary obese rats in this study reflects adipocyte hyperplasia since there is no evident hypertrophy.

Administration of d-fenfluramine for 4 weeks significantly reduced inguinal adipocyte diameters in dietary obese and unoperated Chow-fed rats and, to a lesser extent, in ovariectomized rats, but had no effect on the diameters of inguinal adipocytes of estradiol-treated rats. The effect of d-fenfluramine treatment was to reduce the mean inguinal adipocyte diameters of the dietary obese and unoperated Chow-fed rats by approximately 45%. Fenfluramine had no effect on the mean adipocyte diameter of the estradiol-treated rats; therefore, after 4 weeks of chronic administration, the mean adipocyte diameters of dietary obese and unoperated Chow-fed rats were smaller than adipocyte diameters of the estradiol-treated rats.

By 2 weeks after the termination of d-fenfluramine treatment, the mean inguinal adipocyte diameters of ovariectomized rats had returned to untreated control levels. Mean adipocyte diameters of dietary obese and unoperated Chow-fed rats that had received d-fenfluramine had partially recovered within 2 weeks, but remained below control levels.

In Experiment 2, both dietary restriction and d-fenfluramine treatment reduced mean inguinal adipocyte diameter in all three

condition groups. The mean adipocyte diameter in d-fenfluramine-treated rats in the DIO and CHOW groups was less than that of rats in those groups that had received dietary restriction, but the adipocyte diameter of diet-restricted rats in the OVX group was less than that of d-fenfluramine-treated rats in that group.

Two weeks after the return to ad libitum feeding the diameters of inguinal adipocytes of dietary obese and unoperated Chow-fed rats that had been diet-restricted had not returned to control levels. The inguinal adipocyte diameters of d-fenfluramine-treated rats killed after a 2-week drug-free period had partially recovered, but remained significantly greater than adipocyte diameters of rats killed at the end of the treatment period.

The similarity in adipocyte diameters of untreated control rats in the obese OVX and DIO condition groups and the CHOW group indicates that the increased adiposity of obese ovariectomized and dietary obese rats in this study reflects adipocyte hyperplasia since there is no evident hypertrophy. The time course for development of adipocyte hyperplasia appears to be of critical importance in assessing the long-term effects of overfeeding and weight changes subsequent to changes in diet. Overfeeding for a period of time that is sufficient to allow hyperplasia to develop appears to have long-lasting or permanent effects on body weight. In humans or experimental animals with increased

adipocyte number, dietary restriction or anorectic drug treatment may decrease the amount of lipid stored in existing adipocytes; however, when storage is reduced to a given level, further reductions become increasingly difficult. When dietary restriction is eased, or drug treatment terminated, adipocytes will not have decreased in number and may simply refill with time. In the present experiment, inguinal adipocyte diameters of d-fenfluramine-treated rats had partially recovered toward untreated control levels, but remained low in diet-restricted rats. Perhaps the 2-week posttreatment period did not allow sufficient time to see more complete reversals of adipocyte size.

Brown adipocyte diameters were not significantly different among the four dietary or surgical condition groups and d-fenfluramine treatment had no effect on the size of these adipocytes. In Experiment 2, neither dietary restriction nor d-fenfluramine had a significant effect on adipocyte diameters in IBAT.

Total IBAT pad weights were not measured in these rats; however, during the tissue dissections, it was obvious that IBAT pads were much larger in ovariectomized and dietary obese rats and were very small in estradiol-treated rats compared to unoperated Chow-fed rats. This may be related to the long-term exposure to the procedures used to induce obesity. With short-term feeding of a cafeteria diet, interscapular brown adipocytes increase in size (Tulp, 1981). With relatively long-term feeding of a similar

diet, cell size may decrease, but cell number increases (Triscari et al., 1985; Tulp, 1981).

The relative increase in IBAT weight that occurs subsequent to ovariectomy or diet-induced obesity has been associated with increased thermogenesis and metabolic activity (Himms-Hagen et al., 1981). Alternatively it has been suggested that the increased IBAT weight is the result of excess lipid storage in this tissue (Hervey and Tobin, 1983). The failure of d-fenfluramine to reduce brown adipocyte diameters in cases where it reduced white adipocyte diameters and body weight does not distinguish between the two possibilities; however, it does imply that if the increased IBAT weight is due primarily to increased lipid storage, that this store is less susceptible to the actions of d-fenfluramine on lipid storage.

Effects of d-Fenfluramine on Brain 5-HT and DA

The actions of fenfluramine on brain 5-HT have been studied extensively. Until recently, the majority of behavioral studies have assumed that observed drug effects were primarily, if not exclusively, mediated by brain 5-HT. Blundell (1977, 1979) has published reviews on the involvement of brain 5-HT in the regulation of feeding behavior. The precise nature of this role and the pathways involved have not been defined; however, it is believed to be generally inhibitory.

Fenfluramine competitively inhibits [^3H]5-HT reuptake by brain synaptosomes and stimulates its release from vesicular storage sites (Fuxe et al., 1975; Garattini et al., 1979). Thus the initial effects of fenfluramine administration presumably result in enhanced central 5-HT transmission.

Chronic administration of moderate dosages of d,l-fenfluramine (≤ 10 mg/kg/day) and acute administration of higher dosages of d- or d,l-fenfluramine are associated with decreased brain levels of 5-HT and 5-HIAA in rats (Duhault and Verdavainne, 1967; Duhault et al., 1979, 1981; Garattini et al., 1979). Depletion of 5-HT begins within the first 1-2 hours of drug administration and is followed by decreases in 5-HIAA (Duhault and Verdavainne, 1967; Duhault et al., 1980; Fuller et al., 1978). This suggests that 5-HT is depleted due to increased release without a compensatory increase in synthesis.

Decreases in synthesis are found as early as 2 hours after administration of 10 mg/kg d,l-fenfluramine (Rowland and Carlton, 1986) and may last up to 8 days after a single dose of 15 mg/kg d,l-fenfluramine (Clineschmidt et al., 1978). The ratio of 5-HIAA/5-HT is initially increased (Fuller et al., 1978; Orosco et al., 1984) which further suggests increased neuronal activity. It is not clear whether this increased activity is sustained throughout the period of drug administration.

Decreases in whole brain 5-HT produced by high doses of d,l-fenfluramine are still evident at one month after acute administration (Clineschmidt et al., 1978) although most of the drug is cleared within 24 hours (Garattini et al., 1979). This long-lasting depletion may be due to a neurotoxic action on the nerve terminal (Fuller, 1981).

In the present experiment, the effects of continuous administration of 3 mg d-fenfluramine/kg/day via osmotic minipump on concentrations of 5-HT and 5-HIAA and the ratio of 5-HIAA/5-HT in rat telencephalon and hypothalamus were measured. It was hypothesized that depletions in brain 5-HT reported in previous animal studies might be the result of administering the drug by injections that cause an acute peak in the drug concentration in brain followed by a rapid decline.

Unlike administration of d- or d,l-fenfluramine by intermittent injections, continuous delivery by osmotic minipump for 4 weeks at a dosage of 3 mg/kg/day had no significant effects on the concentrations of 5-HT or 5-HIAA or on the ratio of 5-HIAA/5-HT in the telencephalon. There was no effect of d-fenfluramine treatment on telencephalic 5-HT that was evident at 2 weeks after the termination of treatment.

There were significant increases in the concentrations of 5-HT and 5-HIAA in the hypothalamus, but the ratio of was not significantly affected. Two weeks after the termination of

d-fenfluramine treatment, the concentration of 5-HT in the hypothalamus had returned to control levels.

These results strongly support the hypothesis that depletions of brain 5-HT are an artifact of the mode of administration and the metabolic profile of d-fenfluramine in rats. Using a treatment regimen designed to more closely resemble clinical regimens, chronic administration of d-fenfluramine, at a dose that produced significant reductions in body weight, did not deplete brain 5-HT. The increase in hypothalamic 5-HT and 5-HIAA concentrations after 4 weeks of d-fenfluramine administration was not expected. Perhaps with steady, relatively low concentrations of d-fenfluramine, increased release is accompanied by increased synthesis. The lack of change in the ratio of 5-HIAA/5-HT, insofar as this reflects 5-HT turnover, implies that any changes in release and reuptake are accompanied by changes in synthesis and metabolism so that actual turnover is not significantly changed. The effects of d-fenfluramine on brain 5-HT release, reuptake or binding were not directly measured in the present experiment; therefore, it is not possible to draw definitive conclusions on the effect of this treatment regimen on brain 5-HT activity. It is clear however, that this treatment regimen did not deplete brain 5-HT.

The finding that continuously administered d-fenfluramine sustained suppression of food intake and body weight, but did not deplete brain 5-HT has important clinical implications. One of

the major arguments against the use of fenfluramine in humans has been the possibility of damage to brain 5-HT neurons. In showing that a treatment regimen designed to closely resemble a clinical therapy regimen does not deplete brain 5-HT, a number of issues have been raised that seriously question the applicability of many previous neurochemical studies to the clinical situation.

This also may explain some of the disparity between animal and clinical studies. If indeed tolerance to fenfluramine is due largely to depletions of brain 5-HT, the failure of human subjects, as well as animals in this study, to develop complete tolerance may be explained by the fact that brain 5-HT is not depleted.

This finding also has serious implications for studies on the peripheral actions of d-fenfluramine, many of which are thought to be mediated by 5-HT. Changes seen in these parameters with chronic, compared to acute, fenfluramine may be the result of 5-HT depletion by high doses or concentrations that would not occur clinically.

In Experiment 2, the effects of d-fenfluramine treatment on concentrations of 5-HT, 5-HIAA, DA and DOPAC in rat telencephalon and hypothalamus were compared to dietary restriction and untreated control values. Neither continuous delivery of d-fenfluramine by osmotic minipump nor dietary restriction for 4 weeks affected the concentration of 5-HT in the telencephalon. Dietary

restriction produced a slight, but statistically significant, decrease in the concentration of 5-HIAA and in the ratio of 5-HIAA/5-HT in the telencephalon. The effects of dietary restriction or d-fenfluramine treatment were reversed by the end of the 2-week posttreatment period except that the ratio of 5-HIAA/5-HT in the telencephalon of rats that were diet-restricted remained decreased after 2-weeks of ad libitum feeding.

The decrease in 5-HIAA/5-HT seen with dietary restriction is consistent with the proposed role of 5-HT as a mediator of satiety (e.g. Blundell, 1979): thus "hungry" animals would have lowered 5-HT activity. The majority of previous studies on the role of brain 5-HT and feeding have utilized procedures that alter brain 5-HT concentrations (either increased or decreased) and then measured effects on food intake. There is considerable variability in the results and conclusions of studies on brain 5-HT and appetite; however, the concensus appears to favor a role for 5-HT in mediating or enhancing satiety. Some, but not all procedures that decrease brain concentrations of 5-HT cause increased food intake and body weight and some, but not all, pharmacological manipulations that appear to increase brain 5-HT activity can decrease food intake and body weight (Blundell, 1979).

Peripheral Actions of d-Fenfluramine.

The present experiments provide evidence against a singular brain 5-HT mechanism in the mediation of d-fenfluramine's actions

on food intake and body weight. The hypothesis that d-fenfluramine is more effective in producing sustained weight loss in animals with a higher percentage body fat was confirmed also. The actions of d-fenfluramine on food intake and body weight in these experiments imply that the compound has antiobesity actions other than appetite suppression. Prolonged anorexia and decreased weight gain occurred in rats made obese by feeding a palatable and varied diet. Enhanced and prolonged weight loss occurred in obese ovariectomized rats whereas food intake and body weight of lean ovariectomized, estradiol-treated rats were not affected by d-fenfluramine treatment, even acutely.

Peripheral actions of fenfluramine may be relevant to its antiobesity actions and may also help explain possible differences in drug efficacy in lean and obese animals. The relevance of any of the central or peripheral actions of fenfluramine to appetite suppression and weight loss is not completely clear. When various actions are viewed in isolation or when effects of single doses and high concentrations of drug are extrapolated to explain clinical actions, the results may seem confusing and contradictory. Only by putting together the series of actions as they occur within a relevant treatment model is it possible to determine the exact nature of the factors and interactions important to appetite regulation and weight loss.

As discussed previously, a productive approach to understanding the antiobesity effects of d-fenfluramine is to examine possible drug actions that may affect body weight either in relation to decreased food intake or in apparent absence of major effects on food intake. Several of the possible antiobesity actions of d-fenfluramine were examined in this regard.

In Experiment 1, the effects of chronic administration of 3 mg d-fenfluramine/kg/day (via osmotic minipump) on peripheral metabolism were examined in groups of rats that were killed either at the end of the 4-week treatment period or after a 2-week drug-free posttreatment period and in untreated control rats from the four dietary or surgical condition groups: OVX, EB, DIO and CHOW.

In Experiment 2, these actions were examined further in order to determine the extent to which they may be independent of reduced food intake or body weight. The peripheral effects of 4 weeks of d-fenfluramine administration via osmotic minipump were compared to the peripheral effects of 4 weeks of dietary restriction that allowed rats access to 75% of their baseline caloric intakes and these two treatments were compared to untreated control values. These peripheral effects were measured in rats from the OVX, DIO and CHOW condition groups killed at the end of the 4-week drug or restricted diet treatment. Additional groups of rats in the DIO and CHOW conditions were killed after a 2-week drug-free, ad libitum feeding period.

Effects of d-Fenfluramine on Thermogenesis

The activity of K^+ -nitrophenylphosphatase (NPPase) is an index of the activity of the enzyme $(Na^+-K^+)ATPase$ and hence reflects cellular activity (Swann, 1984a, 1984b). The activity of NPPase was measured in interscapular brown adipose tissue (IBAT) and in gastrocnemius muscle of d-fenfluramine-treated and untreated control rats from each of the four dietary or surgical conditions. These tissues were selected because of previously reported effects of d,l-fenfluramine on the thermogenic efficiency of brown adipose tissue and effects of d-fenfluramine on the cost of muscular activity. It was hypothesized that if d-fenfluramine, in a clinically relevant dose, acts to decrease body weight by increasing energy burning in brown adipose tissue or by increasing the cost of muscular activity, then these actions may be reflected in changes in the activity of NPPase in those tissues. It was further hypothesized that differences in enzyme activity may be associated with obesity induced by diet or ovariectomy and that NPPase activity in obese rats might respond differentially to d-fenfluramine treatment thus offering an explanation for differences in the drug's effects on body weight and food intake in the various dietary or surgical condition groups.

The activity of NPPase in BAT of ovariectomized rats was low compared to control rats in the other condition groups and was less than 25% of control values from unoperated Chow-fed rats.

Administration of d-fenfluramine increased NPPase activity in BAT of ovariectomized rats to a level comparable to the other groups. Fenfluramine also increased NPPase activity in unoperated Chow-fed rats by 45% but did not significantly affect the activity of this enzyme in dietary obese or estradiol-treated rats.

By the end of the 2-week drug-free period, d-fenfluramine-induced increases in NPPase activity in BAT were completely or partially reversed. In ovariectomized rats, the increased NPPase activity associated with d-fenfluramine administration was not reversed completely within 2 weeks, but was significantly lower than NPPase activity measured at the termination of drug treatment.

The changes in activity of NPPase in BAT were consistent with changes in body weight in all except dietary obese rats. The activity of NPPase was low in ovariectomized rats and was substantially increased by chronic d-fenfluramine treatment. The low NPPase activity in untreated ovariectomized rats was associated with obesity that was not maintained by increased food intake. Administration of d-fenfluramine caused a substantial weight loss and increased NPPase activity. Fenfluramine treatment did not increase NPPase activity BAT of dietary obese rats. Estradiol administration also increased NPPase activity in BAT of ovariectomized rats, but d-fenfluramine treatment did not further increase NPPase activity in estradiol-treated rats and was not effective in causing weight loss in those rats.

Long-term feeding of a cafeteria diet may cause changes in NPPase activity in BAT so that it is more difficult to affect enzyme activity. Thus d-fenfluramine increased NPPase activity in Chow-fed, normal weight rats and ovariectomized rats with low control levels of NPPase, but was not effective in increasing NPPase activity in rats that may have alterations in enzyme activity caused by prolonged stimulation of NPPase at some point. Long-term stimulation of this indicator of ATPase activity may lead to changes in beta-adrenergic activity or in sympathetic nervous system activity and subsequent desensitization to stimulation by d-fenfluramine.

The low levels of NNPase activity in ovariectomized rats are consistent with reports of lowered thermogenic activity in ovariectomized rats that is dependent upon sympathetic nervous system activity and is reversed by administration of estradiol benzoate (Bartness and Wade, 1984). The d-fenfluramine-stimulated increase in BAT thermogenesis in ovariectomized and unoperated Chow-fed rats may be related to increased sympathetic activity since acutely administered fenfluramine has mild sympathomimetic effects (Lake et al., 1979) that may be 5-HT mediated (Stajarne and Schapiro, 1959). In the current study, d-fenfluramine administration did not brain deplete 5-HT; therefore, the chronic treatment effects seen here may be similar to acute effects observed with higher doses.

In Experiment 2, there were no significant effects of dietary restriction on the activity of NPPase in BAT of rats killed at the end of the 4-week treatment period or of rats killed after a 2-week ad libitum feeding posttreatment period.

The effects of d-fenfluramine treatment on NPPase in BAT were consistent with its increased efficacy in producing weight loss in ovariectomized rats. Dietary restriction and weight loss have been associated with decreased thermogenesis in BAT under some conditions. Perhaps one reason that dietary restriction was so effective in reducing body weights of ovariectomized rats in the present experiment is related to the failure to decrease the already low NPPase activity in brown adipose tissue of ovariectomized rats. Conversely the effectiveness of d-fenfluramine treatment in reducing body weights of ovariectomized rats in the absence of decreased food intake may have been related to the stimulation of NPPase activity of BAT.

Swann (1984a) reported diet-induced changes in activity of the enzyme $(\text{Na}^+ - \text{K}^+) \text{ATPase}$ in BAT and muscle that appeared to be regulated by beta-adrenergic receptors. Cafeteria feeding increased ATPase activity and this activity remained elevated with return to regular diet. The animals in that experiment increased their caloric intake by 80%, but did not gain weight relative to controls. Food deprivation resulted in decreased ATPase activity that persisted upon refeeding. During the period of refeeding,

rats gained weight approximately 3-times faster than nondeprived controls. Certainly the results of the present experiment do not support this notion.

The activity of NPPase in gastrocnemius muscle was decreased in untreated ovariectomized rats to approximately the same extent as enzyme activity in BAT (<25% of CHOW control value). The NPPase activity of dietary obese rats was also reduced, although to a lesser extent than in ovariectomized rats (55% of CHOW control value). Activity of muscle NPPase in estradiol-treated control rats was increased by approximately 39% over control values from the CHOW group. This dramatic difference between muscle NPPase activity of obese ovariectomized and estradiol-treated rats may be related to differences in activity levels between the two groups. Ovariectomized rats have decreased activity levels compared to unoperated female rats and administration of estradiol increases activity (Wade and Gray, 1979). The markedly low levels of NPPase activity in both BAT and muscle of ovariectomized rats is a probable explanation, at least in part, for the obesity in these rats in the absence of increased food intake.

Administration of d-fenfluramine for 4 weeks increased the activity of muscle NPPase in ovariectomized rats by approximately 47%, but the increase was not sufficient to raise enzyme activity in those rats to control levels of unoperated Chow-fed rats.

Administration of d-fenfluramine had no significant effect on muscle NPPase activity in rats from the other dietary or surgical condition groups compared to untreated controls in those groups; thus, enzyme activity in d-fenfluramine-treated, dietary obese rats remained below the level of activity in unoperated Chow-fed control rats. Perhaps the pronounced effects of d-fenfluramine on NPPase activity in ovariectomized rats and the relative lack of effectiveness in dietary obese rats is one of the reasons for the greater efficacy of d-fenfluramine in producing weight loss in ovariectomized rats compared to the dietary obese and other groups although the hypophagic effects were more obvious in dietary obese rats.

Even and Nicolaidis (1986) have reported that acutely administered d-fenfluramine decreases the efficiency of locomotor activity in rats but has no effect on basal metabolism. Rowland (1986) found no difference in the effects of d,l-fenfluramine on body weights of exercising versus sedentary hamsters. The failure of d-fenfluramine to increase NPPase activity in gastrocnemius muscle of any but ovariectomized rats does not appear to be consistent with the increased cost of muscular activity in normal weight rats (Nicolaidis and Even, 1986); however, those studies used a higher drug dose (7.5 mg/kg). This further implies that ovariectomized rats are more sensitive to the effects of d-fenfluramine.

At the end of the 2-week drug-free period, NPPase activity in gastrocnemius muscle of ovariectomized rats that had been treated previously with d-fenfluramine had returned to control levels.

In Experiment 2, 4 weeks of dietary restriction decreased muscle NPPase activity substantially (20-50%) in all three dietary or surgical condition groups. Dietary restriction decreased NPPase activity whereas d-fenfluramine treatment tended to increase enzyme activity. The decrease in NPPase activity was reversed by a 2-week period of ad libitum feeding in rats from the unoperated Chow-fed rats, but not in dietary obese rats. There was no significant effect of d-fenfluramine treatment on muscle NPPase activity of rats in dietary obese or unoperated Chow-fed rats that were killed at the end of drug treatment or after a 2-week drug-free period.

It was hypothesized that significant changes in thermogenesis would be accompanied by changes in body temperature. In Experiment 3, colonic temperatures of rats treated with d-fenfluramine (3 mg/kg/day via osmotic minipump) and untreated controls were measured in the four dietary or surgical condition groups: obese ovariectomized (OVX); ovariectomized, estradiol-treated (EB); dietary obese (DIO) and unoperated Chow-fed (CHOW).

Changes in thermogenesis are accompanied by changes in body temperature or in heat exchange. Levin et al. (1983) found that rats with diet-induced obesity showed impaired thermogenic

responses when exposed to cold. The lower body temperatures of ovariectomized rats in the present were accompanied by lower levels of NPPase activity in BAT and increased body temperatures in dietary obese rats were associated with increased NPPase activity in BAT. The thermogenic response of dietary obese rats was not obviously impaired in the present experiment in that during cold exposure, their colonic temperatures did not drop significantly lower than those of rats in other groups; however, the total decrease in colonic temperatures of dietary obese rats in response to cold was greater than in other groups because their temperatures were significantly higher at normal room temperature (25 °C).

In the present study, d-fenfluramine treatment tended to decrease colonic temperatures, but not significantly, at either 25 °C or 4 °C. Previous reports on the effects of fenfluramine on body temperature are variable, but generally slight decreases are reported. Pawlowski et al. (1980) and Sugrue (1981, 1984) reported decreases of 1-2 °C in rectal temperatures of d,l-fenfluramine-treated rats. This effect appeared to be 5-HT dependent. It is possible that the decreases in body temperature reported in that study were due to increased heat loss since 5-HT is a peripheral vasodilator. A similar mechanism may explain the failure to find increased body temperatures in rats that had increased thermogenic activity in BAT.

Effects of d-Fenfluramine on Lipoprotein Lipase (LPL) Activity

Lipoprotein lipase is the principle enzyme in the storage of lipids in adipose tissue and may be sensitive to changes in diet and body weight. Adipocyte LPL is related to cell size (Hietanen and Greenwood, 1977; Brunzell, 1979) and is elevated in several animal models of obesity as well as in a population of obese and formerly obese humans. The increase in LPL activity may occur in pre-obese animals prior to hyperphagia (Boulangue et al., 1979) and development of hyperinsulinemia (Turkenkopf et al., 1982). Obese humans have increased LPL activity per adipocyte but no difference in activity per gram tissue (Guy-Grand and Bigorie, 1975; Lithell and Boberg, 1978; Pykalisto et al., 1975; Taskinen and Nikkila, 1977). Whether or not this indicates a functional increase in activity is not clear.

The activity of retroperitoneal LPL was lower in dietary obese rats and higher in ovariectomized rats than in unoperated Chow-fed control rats. Four weeks of chronic d-fenfluramine administration significantly reduced retroperitoneal LPL activity in rats from each of the four dietary or surgical condition groups, including estradiol-treated rats. The reductions LPL activity were substantial, ranging from a 38% decrease in dietary obese rats to a 78% decrease in ovariectomized rats. At the end of the 2-week drug-free period, LPL activity in retroperitoneal adipose tissue of dietary obese and estradiol-treated rats that

had received d-fenfluramine had returned to control levels. Lipoprotein lipase activity in retroperitoneal adipose tissue of ovariectomized and unoperated Chow-fed rats had been affected by d-fenfluramine treatment to a greater extent and had not returned to control levels within 2 weeks of the termination of drug treatment.

Lipoprotein lipase activity in inguinal WAT was also elevated in ovariectomized rats compared to rats in the other dietary or surgical condition groups; however, LPL activity in dietary obese rats was not significantly different from that of estradiol-treated rats or unoperated Chow-fed rats. Administration of d-fenfluramine for 4 weeks had no significant effect on inguinal LPL activity in any of the dietary or surgical condition groups.

Inguinal LPL activity was not closely correlated with inguinal adipocyte diameter in this study. Lipase activity was approximately 3-times greater in ovariectomized rats than in dietary obese or unoperated Chow-fed rats although inguinal adipocyte diameter was not significantly different among the three groups. Furthermore, inguinal adipocyte diameter was reduced in estradiol-treated rats compared to unoperated Chow-fed rats, but inguinal LPL activity was not significantly different among these groups. This is additional evidence for the suggestion that the relatively long-term obesity produced in this experiment resulted in adipocyte hyperplasia.

The activity of LPL in IBAT was higher in both ovariectomized and dietary obese rats and was lower in estradiol-treated rats compared to unoperated Chow-fed rats. Administration of d-fenfluramine for 4 weeks had no significant effect on LPL activity in BAT and there were no significant differences in the activity of LPL in IBAT samples taken from previously d-fenfluramine-treated rats killed after a 2-week drug-free period compared IBAT samples taken at the termination of drug treatment or untreated controls. If increased LPL activity in BAT of ovariectomized and dietary obese rats resulted in hyperplasia, this could partially explain the increased size of the IBAT pads in obese rats.

In the present study, LPL activity in the three types of adipose tissue examined was approximately 3-times higher in ovariectomized rats than in unoperated Chow-fed rats and administration of 2 ug estradiol benzoate/day reversed this effect of ovariectomy. The substantially elevated LPL activity in ovariectomized rats relative to other groups is consistent with previous reports of increased LPL activity in ovariectomized rats (Steingrimsdottir et al., 1980; Wade and Gray, 1978) and decreased activity with estradiol replacement has been reported also (Gray and Wade, 1981).

Lipoprotein lipase activity was decreased by approximately 50% in retroperitoneal white and interscapular brown, but not in

inguinal white, adipose tissue of dietary obese rats compared to unoperated Chow-fed rats. Previous reports on changes in LPL activity with diet-induced obesity are inconsistent, both increases and decreases have been reported. The differences in the diet-induced change in LPL activity in the different adipose tissues are consistent with previous reports of tissue differences in the responsivity of LPL to changes in diet.

The effects of d-fenfluramine on LPL activity have not been previously reported. In the present study, d-fenfluramine markedly decreased LPL activity in retroperitoneal adipose tissue of rats in each of the four dietary or surgical condition groups, but had no significant effect on LPL activity in inguinal or brown adipose tissues.

In some situations, changes in LPL activity are associated with weight changes and with extended dieting, LPL activity may be increased, an effect that would have important implications for long term weight regulation (Schwartz and Brunzell, 1981). Lipoprotein lipase has been reported to be sensitive to changes in diet and body weight. Adipocyte LPL is related to cell size (Hietanen and Greenwood, 1977; Brunzell, 1979) and is elevated in several animal models of obesity as well as in a population of obese and formerly obese humans. The rate of weight loss in dieting humans is inversely correlated with progressive changes in LPL activity. Thus during the initial rapid weight loss that

accompanies a hypocaloric diet, there is no change in IPL activity; however, with continued caloric restriction, IPL activity is increased, weight loss slows and the individual often reports increasing discomfort with the restricted diet (Schwartz and Brunzell, 1981).

In Experiment 2, 4 weeks of dietary restriction had no significant effect on IPL activity in any of the tissues examined. The correlation between changes in IPL activity and changes in food intake and body weight has not been found in universality; however, increased IPL activity might have been predicted from the profile of hyperinsulinemia and hypercorticism associated with dietary restriction in the present study.

Effects of d-Fenfluramine on Carbohydrate and Lipid Metabolism

The fasted (12-18 hours) daytime plasma insulin concentrations of obese ovariectomized rats, estradiol-treated rats and dietary obese rats were elevated substantially compared to unoperated Chow-fed rats. Administration of d-fenfluramine for 4 weeks had no significant effect on plasma insulin concentrations of rats in the four dietary or surgical conditions and there were no significant differences in plasma insulin concentrations of d-fenfluramine-treated rats killed after a 2-week drug-free period compared to insulin concentrations of rats killed at the end of the treatment period or untreated control rats.

The marked hyperinsulinemia associated with ovariectomy or dietary obesity is consistent with previous reports (eg. Triscari et al., 1985). Hyperinsulinemia in estradiol-treated rats has also been reported: exogenously administered or endogenously secreted estradiol increase plasma insulin levels (Bailey and Matty, 1972; Matute and Kalkhoff, 1973). Increased insulin levels in obese ovariectomized rats and estradiol-treated rats may reflect pancreatic synthesis and tissue sensitivity to insulin. Effects of estrogen on food intake and body weight are not critically dependent upon insulin.

Hyperinsulinemia is commonly found in a majority of obese animal models and in humans with longstanding obesity. The result of persistent hyperinsulinemia associated with obesity may be the development of insulin receptor insensitivity and Type II diabetes mellitus which constitutes a major health risk for the chronically obese.

In Experiment 2, neither chronic d-fenfluramine treatment nor dietary restriction for 4 weeks had significant effects on plasma insulin concentrations in any of the groups of rats. Both dietary restriction and d,l-fenfluramine treatment have been reported to improve insulin sensitivity. Perhaps the length of the treatment time was not sufficient for changes in plasma insulin concentrations to be manifested.

Dietary obese rats were hypoglycemic after being fasted overnight (12-18 hours) prior to the time they were killed. Four weeks of d-fenfluramine administration or dietary restriction elevated fasting glucose concentrations of dietary obese rats to normal (100-125 mg/dl) levels, but had no significant effect on glucose concentrations of rats in the other dietary or surgical condition groups. The hypoglycemia in dietary obese rats was not expected; however, the metabolic profile in these rats was marked by hyperinsulinemia and relatively high circulating corticosterone concentrations. The combination of hyperinsulinemia and hypercorticism is found in both humans and in some animal models of longstanding obesity. Both dietary restriction and d-fenfluramine administration brought fasted plasma glucose concentrations within the normal range although both treatments were associated with continued hyperinsulinemia and actual increases in plasma corticosterone concentrations. The increased corticosterone concentrations would be expected to raise plasma glucose concentrations by stimulating gluconeogenesis. Insulin receptor sensitivity may also have been improved even though fasting plasma insulin concentrations were not obviously decreased; however, glucocorticoids can decrease insulin sensitivity and this may be the reason hyperinsulinemia was not improved by d-fenfluramine treatment or dietary restriction in the present study.

Hyperinsulinemia has been associated with elevated plasma lipids. Elevated plasma free fatty acids can inhibit insulin clearance (Smith, 1985) and also glucose uptake. With chronic d,l-fenfluramine treatment, free fatty acid levels are correlated with drug levels. Thus elevated free fatty acids may partially explain the increase in plasma glucose concentrations seen in dietary obese rats after 4 weeks of d-fenfluramine treatment.

Although fasting plasma insulin and glucose concentrations in Experiments 1 and 2 were not affected by d-fenfluramine treatment, it was hypothesized that glucose tolerance may be a more sensitive index of the effects of d-fenfluramine on carbohydrate metabolism. Previously reported effects of d,l-fenfluramine on carbohydrate metabolism in normal weight laboratory animals and humans include increased glucose uptake (Butterfield and Whicelow, 1968) and improved glucose tolerance (Doar et al., 1979; Duhault et al., 1979; Whicelow and Butterfield, 1970).

In Experiment 3, the effects of administration of 3 mg d-fenfluramine/kg/day (via osmotic minipump) on plasma concentrations of insulin were measured under basal nighttime conditions on the third day of drug administration in rats from the four dietary or surgical condition groups: OVX, EB, DIO and CHOW. Glucose tolerance was estimated from plasma glucose concentration measured at 20 minutes after a 25% glucose load.

With regard to plasma insulin concentrations, the results of the Experiment 3 confirmed those of Experiments 1 and 2. Night-time insulin levels were elevated in ovariectomized rats, estradiol-treated rats and dietary obese rats. In Experiments 1 and 2, insulin concentrations of dietary obese rats were slightly, but significantly, less than those of obese ovariectomized and estradiol-treated rats; however, insulin concentrations were not significantly different among those groups in Experiment 3.

Basal nighttime glucose concentrations of dietary obese rats tended to be higher, although not significantly, than plasma glucose concentrations of rats in the other dietary or surgical condition groups. This suggests that the hypoglycemia seen in Experiments 1 and 2 was the result of a reaction to the overnight fast (12-18 hours).

Although fasting plasma glucose concentrations (Experiments 1 and 2) were not affected by d-fenfluramine treatment, glucose tolerance was improved by d-fenfluramine treatment in estradiol-treated rats and unoperated Chow-fed rats but not in ovariectomized or dietary obese rats. It is possible that with continued administration of d-fenfluramine, glucose tolerance would have improved in the obese rats in this study. The improvement in glucose tolerance seen with d-fenfluramine treatment is consistent with previously reported effects of clinical doses of d,l-fenfluramine (Doar et al., 1979; Duhault et al., 1979; Whichelow and Butterfield, 1970).

Plasma triglyceride concentrations of overnight fasted ovariectomized or dietary obese and unoperated Chow-fed rats were similar and there were no significant effects of dietary restriction or d-fenfluramine treatment on plasma triglyceride concentrations.

In Experiment 1, plasma corticosterone concentrations were elevated in untreated dietary obese rats, compared to the other dietary and surgical condition groups in this study. Chronic administration of d-fenfluramine substantially elevated plasma corticosterone concentrations of rats in all four dietary or surgical condition groups compared to the corresponding group controls. By 2 weeks after the termination of d-fenfluramine treatment, plasma corticosterone concentrations had returned to control levels.

In Experiment 2, both dietary restriction and administration of d-fenfluramine for 4 weeks produced significant elevations in plasma corticosterone concentrations in rats from all three dietary or surgical condition groups compared to the corresponding group controls. By 2 weeks after the termination of d-fenfluramine treatment or dietary restriction, plasma corticosterone concentrations had returned to control levels.

Previous reports on the effects of d- and d,l-fenfluramine on plasma corticosterone have used high drug concentrations. In those studies, acute d,l-fenfluramine stimulated the release of

adrenal glucocorticoids and catecholamines (Schettini et al., 1979). Brindley (1983) has found similar effects with d-fenfluramine. These effects appear to be mediated by increased hypothalamic 5-HT availability which stimulates release of corticotropin releasing hormone (CRH). The time course of this action is parallel to that of anorexia and tolerance with daily injections of d,l-fenfluramine (Fuller et al, 1981).

The reported effects of chronic injections of high doses of d-fenfluramine on the adrenal hormones are quite different from the acute effects. Basal corticosterone levels are normal and the stress-evoked peaks in plasma corticosterone, catecholamines and free fatty acids are substantially decreased (Brindley et al., 1985). Those effects were seen with a high drug dosage (25 mg/kg/day), but not with a lower a dosage (2.5 mg/kg twice daily).

The elevated plasma corticosterone concentrations found under basal conditions after 4 weeks of chronic administration of 3 mg d-fenfluramine/kg/day via osmotic pump suggest that this method of administration produces a profile of chronic drug effects that are more like the acute effects seen with intermittent injections, particularly where high doses are used. Other results of this study support this notion: brain 5-HT is not depleted and no apparent tolerance develops to weight loss with this treatment regimen. The clinical implications of this are important and the

applicability of previous studies using intermittent administration of high doses of fenfluramine is challenged.

Effects of d-Fenfluramine on Responses to Cold Exposure

There is indirect evidence that fenfluramine has effects on stress hormones that may be related to its antiobesity actions:

- (a) administration of d-fenfluramine decreases the ethanol-induced rise in plasma corticosterone (Brindley et al., 1979);
- (b) d,l-fenfluramine is effective in reversing obesity resulting from overeating induced by either tail pinch (Antelman et al., 1979) or central administration of muscimol (Borsini et al., 1982) both of which are proposed animal models of stress-induced eating;
- (c) glucocorticoid-induced obesity in humans is responsive to d,l-fenfluramine treatment (Cameron et al., 1972; Tomlinson et al., 1975) and
- (d) d,l-fenfluramine may be effective in treating obesity associated with stress-induced eating (Antelman and Caggiula, 1979; Robinson et al., 1985). Acutely, d-fenfluramine stimulates glucocorticoid release and chronically administered d-fenfluramine decreases the stress-induced peak in corticosterone, but only at a very high dose (25 mg/kg) and not at lower doses (Brindley et al., 1985).

The effects of chronic administration of 3 mg d-fenfluramine/kg/day (via osmotic minipump) on plasma concentrations of triglycerides and corticosterone were measured under basal nighttime conditions and after 4 hours exposure to cold (4 °C). Plasma

samples were collected from chronically indwelling jugular catheters under basal conditions on the third day of d-fenfluramine administration and after 4 hours of cold exposure on the fourth day of drug treatment.

There was a cold-induced rise in plasma triglycerides; however, the d-fenfluramine treatment regimen used in this experiment was not effective in reducing the elevated triglyceride concentrations. Previous reports on the ability of fenfluramine to reduce the stress-induced rise in triglycerides found this effect only at high drug doses (Brindley, 1985). In high concentrations, d-fenfluramine decreases circulating triglycerides (Brindley, 1983; Brindley et al., 1985). In those experiments, rats were maintained on a high fat diet and the stressor was a fructose load. It is possible that the type of stress is important: thus d-fenfluramine may reduce the triglyceride rise in response to a metabolic stress, but have a no effect on the triglyceride peak in response to other types of stress.

Plasma corticosterone concentrations were elevated in dietary obese rats compared to untreated control rats in the other dietary or surgical condition groups. Plasma corticosterone concentrations were elevated by cold exposure in all dietary or surgical condition groups. Fenfluramine treatment elevated corticosterone levels in all condition groups at 25 °C, but did not affect the high corticosterone concentrations at 4 °C. The elevation in

plasma corticosterone concentrations induced by d-fenfluramine treatment was comparable to the corticosterone peak induced by 4 hours of cold exposure and both were at, or near, peak levels.

Previously reported effects of chronically administered high doses (25 mg/kg/day) d-fenfluramine are different from acute effects and different from the effects of the chronic treatment regimen used in the present experiments. In previously reported experiments, basal corticosterone levels were normal and the stress-evoked rise in plasma corticosterone, catecholamines and FFA were substantially decreased (Brindley et al., 1985). Those effects were seen with a high drug dosage (25 mg/kg/day), but not with a lower dosage (2.5 mg/kg twice daily).

The d-fenfluramine-induced increase in plasma corticosterone concentrations found in the present experiments is similar to the acute effect of higher doses. This is consistent with a 5-HT mechanism for the d-fenfluramine-stimulated rise in glucocorticoids: in a paradigm in which 5-HT is not depleted, glucocorticoid levels remain elevated. Acutely, d,l-fenfluramine stimulates corticosterone release (Fuller et al., 1981; McElroy et al., 1984; Schettini et al., 1979) and has mild sympathomimetic actions including increased plasma NE (Calderini et al., 1975; Lake et al., 1979). These effects may be mediated in part by central or peripheral 5-HT: exogenously administered 5-HT stimulates the sympathetic nervous system and stimulates release of corticotrophic releasing hormone.

Effects of d- and d,l-Fenfluramine
on Lateral Hypothalamic Self-Stimulation

Both brain DA and brain opioids have been implicated in stress-induced eating and as possible mediators of the rewarding properties of food. It likely that there are significant interactions among brain DA, 5-HT and opioid systems that affect eating behavior.

Electical current delivered via electrodes implanted in the area of the medial forebrain bundle of the lateral hypothalamus will produce both consumatory behaviors and reinforcing effects. Animals readily learn to press a bar in order to deliver intracranial stimulation (ICSS). When food is present, electrical stimulation to this area will induce consumatory behaviors including eating and gnawing.

The effects of injections of different dosages d- and d,l-fenfluramine on the responding of rats trained to press a bar to self-deliver electrical current to the lateral hypothalamus via implanted stainless steel electrodes were assessed. Rats were tested with increasing dosages of d- and d,l-fenfluramine. Both d- and d,l-fenfluramine were effective in suppressing ICSS; however, the racemate was more potent in this regard. The ED₅₀ dosages were 5.5 mg/kg d-fenfluramine and 2.3 mg/kg d,l-fenfluramine.

Hoebel et al. (1986) have reported that d-fenfluramine decreases ICSS responding in rats with electrodes in the area of

the lateral hypothalamus that was also associated with ESB-induced eating and excitation of taste neurons. Those authors concluded that d-fenfluramine inhibits feeding reward and taste reward, and that these effects are mediated by release of 5-HT in the lateral hypothalamus.

The results of the present experiment do not support that hypothesis, but rather suggest that a dopaminergic mechanism is primarily involved in the suppression of ICSS responding by d- and d,l-fenfluramine. Both d- and l-fenfluramine stimulate brain DA turnover (Jori and Dolphini, 1974; Garattini et al., 1975). Although the l-isomer is more potent in this regard, d-fenfluramine is at least as potent as an equal dose of amphetamine (Rowland and Carlton, 1986). This does not rule out the possibility that brain 5-HT or opioids are also involved in this effect of fenfluramine. It does imply that brain 5-HT is not the primary mediator since the d-fenfluramine is more effective in stimulating brain 5-HT activity, but less effective in suppressing ICSS than equivalent doses of d,l-fenfluramine.

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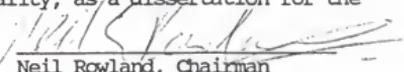
BIOGRAPHICAL SKETCH

Janis Carlton was born May 27, 1954, in Lexington, North Carolina, to Dorothy and Grady Hallman. Her degrees include a Bachelor of Science degree in psychology, received from the Coastal campus of the University of South Carolina in 1981, and a Master of Science with a concentration in psychobiology from the University of Florida in 1983.

Her teaching experience includes three years as a teaching assistant in psychobiology and one semester as an instructor of psychobiology. Her major research experience at the graduate level has been concerned with physiological bases of ingestive behaviors focusing primarily on mechanisms of action and tolerance to appetite suppressant drugs. Her current research projects examine various actions of the anorectic agent d-fenfluramine (Isomeride, Servier Laboratories) which is currently being tested for use in humans.

Following graduation, she will begin postdoctoral training with the goal of pursuing a career in academic research and teaching.

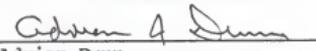
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Professor of Psychology

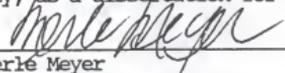
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Robert Cousins
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Adrian Dunn
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This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1987

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